

**THE DEPENDENCE OF DEGRADATION AND RELEASE BEHAVIOR OF  
POLYESTERS ON MONOMER SEQUENCE**

by

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# **THE DEPENDENCE OF DEGRADATION AND RELEASE BEHAVIOR OF POLYESTERS ON MONOMER SEQUENCE**

Jian Li, PhD

University of Pittsburgh, 2012

Although Nature has taught us that sequence control can be used to control macromolecular properties, there is little systematic polymer research connecting sequence with properties. Poly(lactic-*co*-glycolic acid) (PLGA) copolymers are universally recognized as biodegradable polymers that can be used for *in vivo* bioengineering applications. Sequence control of PLGAs may allow the tuning of properties for specific drug delivery or cell scaffolding applications. A series of sequenced PLGAs were prepared and their hydrolysis rates, thermal properties and ability to sequester and deliver rhodamine B (RhB) were investigated. By monitoring both polymer molecular weight and lactic acid release, it was determined that sequenced copolymers hydrolyze more gradually than random copolymers. Data from thermal studies and from the size exclusion chromatography of these samples establish also that they maintain their initial morphology throughout and do not become heterogeneous as do the random controls. Differences in hydrolysis profile were also found for specific sequences. RhB encapsulation and release was also found to depend on sequence—the alternating copolymer had a lower loading capacity but a more gradual release rate than the random copolymer with the same lactic/glycolic unit composition.



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## LIST OF ABBREVIATIONS

Å	angstrom
AcOH	acetic acid
atm	atmosphere
Bn	Benzyl protecting group
brine	saturated aqueous NaCl
Bu	tert-butyl
° C	degrees Celsius
d	doublet
DCC	N-N'-dicyclohexylcarbodiimide
dd	double doublet
DIC	dicyclohexylcarbodiimide
DMAP	dimethylamino pyridine
DPTS	4-(Dimethylamino)pyridinium 4-toluenesulfonate
EtOAc	ethyl acetate
G	Glycolic acid monomer
Hz	hertz
J	coupling constant
kDa	kilodalton

L	Lactic acid monomer
M	molar (moles per liter)
m/z	mass/charge ratio
M <sup>+</sup>	molecular ion
MALDI-ToF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy
mg	milligram
MHz	megahertz
min	minute
mL	milliliter
mmol	millimole
M <sub>n</sub>	number average molecular weight
mol	mole
M <sub>w</sub>	weight average molecular weight
nm	nanometer
NMR	Nuclear magnetic resonance
PDI	Polydispersity
PDLA	Poly(D-lactic acid)
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(L-lactic acid)
PLLGA	Poly(L-lactic-co-glycolic acid)

ppm	parts per million
q	quartet
ref	reference
RhB	rhodamine-B
ROP	ring-opening polymerization
rt	room temperature
s	singlet
Si	<i>tert</i> -butyldiphenylsilyl group
SAP	segmer assembly polymerization
T	temperature
t	triplet
TBAF	tetra-n-butylammonium fluoride
TBDPSi	<i>tert</i> -butyldiphenylsilyl group
T <sub>g</sub>	glass transition temperature
THF	tetrahydrofuran
T <sub>m</sub>	melting transition temperature
δ	chemical shift
λ	wavelength
μ L	microliter

## **PREFACE**

It has been a challenging journey for me to complete my Ph.D. Fortunately, I was advised and helped on my way to my destination. One step before its completion, I would like to take the time to thank all the people who have encouraged, supported and influenced me. You are the great fortune of my life.

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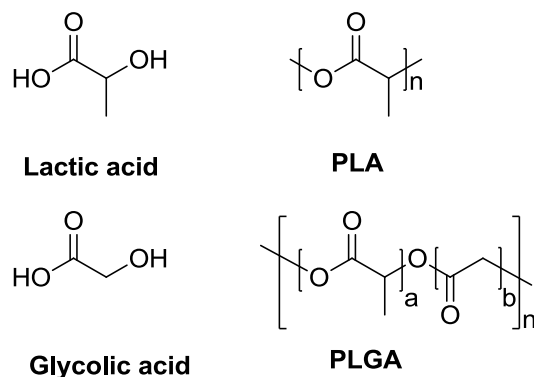
I also thank my proposal committee: Dr. Haitao Liu, who was my mentor, and Doctors Alexander Star and Jill Millstone, who served on my proposal committee. I learned an excellent lesson in the way scientists think about research from them.

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## 1.0 BIODEGRADABLE POLY(LACTIC-CO-GLYCOLIC ACID)S: SYNTHESIS, CHARACTERIZATION AND APPLICATIONS

### 1.1 BACKGROUND

Polyesters are exploited widely in bone tissue engineering and controlled drug release because, for certain monomer combinations, they have been found to be non-toxic, permeable and biodegradable.<sup>1,2</sup> Among the biodegradable polyesters, poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) (Figure 1, L = lactic monomer, G = glycolic monomer), are perhaps the most well-known of the aliphatic polyesters. These polymers, which produce bioassimilable lactic and glycolic acids after hydrolysis,<sup>3</sup> have attracted significant attention for clinical applications such as the replacement of connective tissue and bone<sup>4</sup> and the controlled delivery of drug drugs *in vivo*.<sup>5</sup>



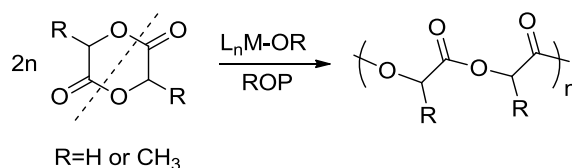
**Figure 1.** Lactic acid, glycolic acid, poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic)acid (PLGA)

PLA and PLGA were introduced several decades ago.<sup>5</sup> Both high molecular weight and low molecular weight polymers have been studied<sup>6-9</sup> and these polymers, especially PLGA, have been widely explored for a variety of biomedical applications.<sup>5</sup> In tissue engineering, for example, by *in vitro* culturing bovine chondrocytes on PLGA scaffolds for 12 weeks, Ma *et al.*,<sup>10</sup> built up artificial cartilage and reported that the thickness of scaffolds increases by 42%. They also found that the compressive modulus and the permeability were comparable to normal bovine cartilage. In another system, Holy *et al.*, seeded pre-cultured rat bone marrow cells on PLGA (75/25 L:G) shaped as a three dimensional scaffold similar to trabecular bone.<sup>11</sup> After culturing for 6 weeks, the cells proliferated in the porous matrix of the polymer scaffold and the formed trabecular bone stabilized osteotomy defect sites in rabbits.

PLGA has also been used in drug delivery systems. Ogura *et al.*, for example, loaded retinal pigment epithelium and the fluorescent dye, rhodamine 6GX, in microparticles of PLGA (75/25 or 50/50 L:G) for *in vivo* drug delivery in the subretinal space of rabbits.<sup>12</sup> The controllable release rate was found to depend on molecular weight and the copolymer composition. The microparticles degraded in the cytoplasm and the implanted retinal sites were not damaged. For topical drug delivery, Jalón *et al.*, studied the distribution of PLGA microparticles in porcine skin and found that the particles were able to reach the epidermis through the stratum corneum in high numbers.<sup>13</sup> This behavior is consistent with the use of PLGA as a sustainable release carrier for transdermic drug delivery.



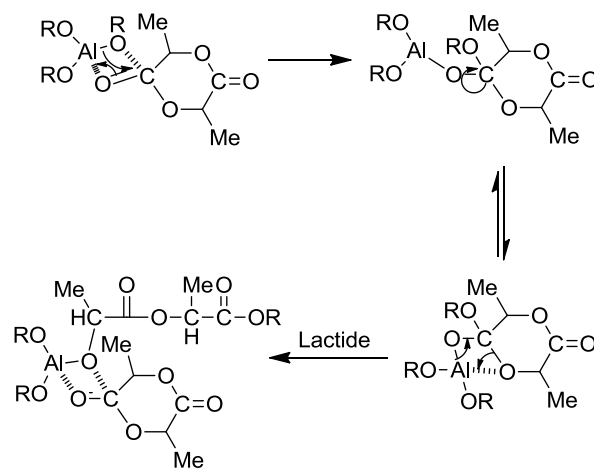
## 1.2 SYNTHETIC APPROACHES TO PLA AND PLGA



**Figure 2.** Ring opening polymerization of cyclic dimers to produce PLA, PGA and/or PLGA

Several synthetic approaches to PLA and PLGA have been reported.<sup>9,14-16</sup> The ring-opening polymerization (ROP) of six-membered cyclic esters which are formed by lactic acid or glycolic acid is the most commonly used (Figure 2).<sup>6,8</sup> Two steps are needed in a typical ROP reaction for making PLA. First, the lactide, which is the cyclic di-ester of lactic acid, is prepared.<sup>17</sup> The synthetic preparation from lactic acid involves the application of heat and vacuum to remove water and form the lactide.<sup>18</sup> The lactide is then thermally opened at 200 °C - 300 °C using a metal or metal oxide catalyst. As there are two stereoisomers for lactic acid, L-lactic acid and D-lactic acid, L,L-lactide, D,D-lactide can be prepared from each stereoisomer and D,L-lactide can be isolated from the product of D,L-lactic acid as starting materials. With these stereoisomers of lactide, PLA with corresponding stereochemistry can be prepared.<sup>6,8,19,20</sup> Dubois *et al.*, using L,L-lactide and D,L-lactide as monomers of ROP and aluminum isopropoxide as initiator, studied the mechanism of polymerization in the solution of toluene at 70 °C or in bulk at 180 °C according to the “coordination-insertion” mechanism shown in Figure 3.<sup>8</sup> Lactide inserts into the Al-O bond and the acyl oxygen bond selectively cleaves, which leaves a new open site on the initiator. With the cyclic insertion of lactides, the chain grows and finally forms PLA polymer with an alcohol and ester end groups. Although the living character of the polymerization allows the molecular weight to be controlled by adjusting the ratio of lactide and initiator, the highest molecular weight obtained under these conditions is 90,000 ( $M_n$ ). Besides aluminum alkoxide,

other metal alkoxide initiators have also been studied in ROP of lactides. Kricheldorf *et al.*, applied potassium, titanium, magnesium, zirconium, zinc and tin alkoxide as initiators and studied the mechanisms of different metals in the polymerization.<sup>21</sup> Stannous (II) chloride and stannous (II) 2-ethylhexanoate (tin octoate) initiators are the most widely used catalysts for the polymerization of lactide used in medical applications<sup>17,19,22</sup> because they both have been permitted as food additives and the tin octoate is efficient for polymerization and preventing racemization of lactide.<sup>18</sup> Organocatalysts such as amines, phosphines, and N-heterocyclic carbenes were also found highly active for ROP of lactide.<sup>23-25</sup> Both amine and N-heterocyclic carbenes catalysts are more active than phosphines catalysts so that less transesterification reactions can be observed from the products.<sup>15</sup> Similar methods are used to prepare polyglycolide (PGA).



**Figure 3.** Mechanism for the ring-opening polymerization of lactide using an aluminum catalyst. Adapted with permission from ref. 8. Copyright (1991) American Chemical Society.<sup>8</sup>

PLGA copolymers, which are more widely used than PLA in drug delivery due to their tunable rates of release and degradation, can also be prepared by the ROP method.<sup>26,27</sup> PLGA copolymers were most commonly synthesized by mixing a pre-determined ratio of lactide and glycolide monomers in bulk or in solvent under vacuum and at high temperature with stannous

octoate as the catalyst.<sup>28</sup> As the glycolide has higher reactivity than lactide under these reaction conditions,<sup>29</sup> the final composition usually has a broader lactic/glycolic ratio than would be expected. The block sequence is random.<sup>30</sup> Alternating PLGA with molecular weight up to 66 kDa has been prepared by condensation polymerization of *o*-(2-bromopropionyl) glycolic acid.<sup>31</sup> By the ring closing reaction of *o*-(2-bromopropionyl) glycolic acid, Dong *et al.*,<sup>14</sup> synthesized D,L-3-methylglycolide monomer. This monomer was then polymerized using ROP to give the racemic alternating PLGA copolymer with molecular weights as high as 54 kDa ( $M_n$ ).<sup>32</sup>

Ring-opening polymerization offers a way to obtain high molecular weight PLA and PLGA for biomedical applications. However, as the lactic acid and glycolic acid both have one carboxylic acid end group and one hydroxyl end group, they can also be polymerized through direct condensation polymerization. The disadvantage of direct condensation polymerization is that the water present in the starting materials and produced from the condensation stabilizes both reactive end groups such that the rate constant is reduced. Several approaches have been studied to conquer this problem. By using azeotropic distillation in the presence of diphenylether and molecular sieves (3 Å) to remove the water in the reaction flask, Ajioka *et al.*, successfully synthesized PLA homo-polymer and PLGA copolymer.<sup>33</sup> Tin was used as the catalyst and the water content during the reaction was controlled below 3 ppm to give PLGA with molecular weights as high as 160 kDa ( $M_w$ ). The disadvantage of this reaction is that the oligo-lactic acid depolymerizes at high temperature, such that scrambling can occur, limiting the access to controlled composition.

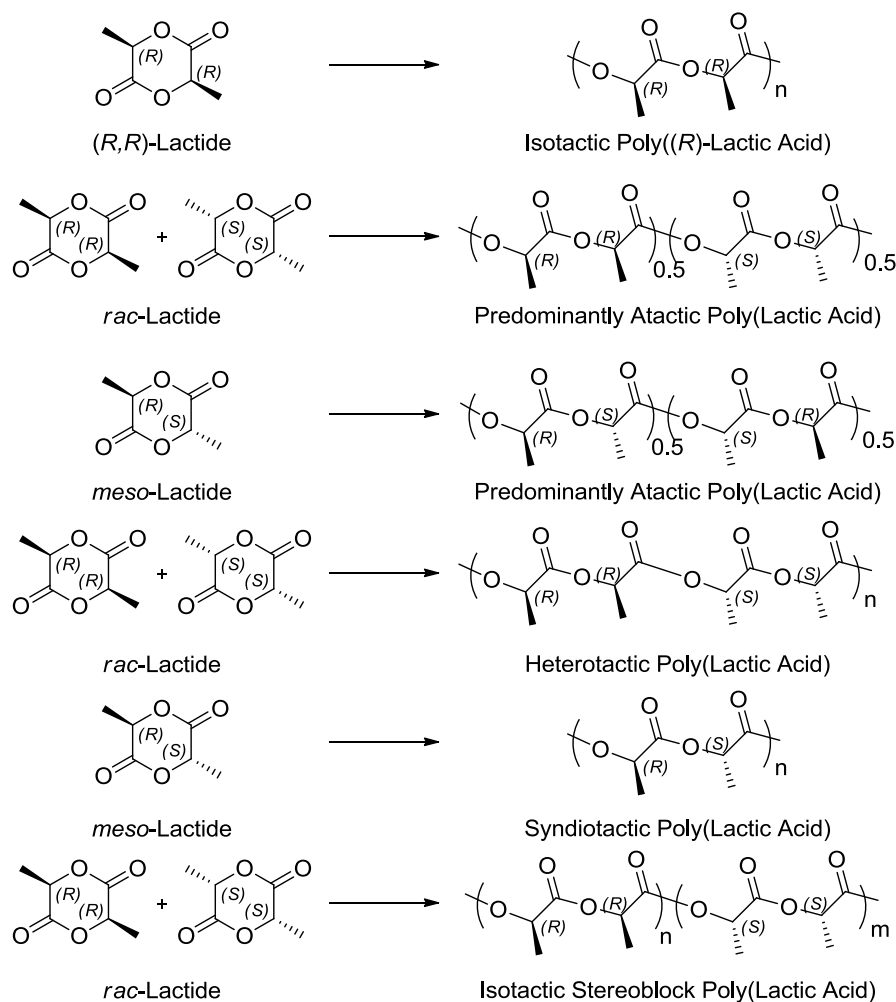
The majority of ROP PLAs and PLGAs are prepared using toxic organometallic compounds as catalysts. Although there is an increasing use of organic catalysts to avoid this problem, others have explored the use of enzymatic catalysts to promote direct condensation polymerization of

lactic and glycolic acids as an alternative. Several enzymes such as *Pseudomonas cepacia* lipase, *Pancreatic porcine* lipase and *Candida antarctica* lipase (Novozyme 435) have been used in the polymerization.<sup>34,35</sup> As the enzymatic catalysts need to work at a specific temperature to exhibit the highest catalyzing ability, in a typical synthesis procedure, lactic acid and glycolic acid monomers were mixed with pre-determined molar ratio and maintained at the selected temperature (usually 60 °C – 80 °C) under vacuum to remove water as byproduct (a solvent with high boiling point was needed). The polymer products, PLA or PLGA, can be dissolved in organic solvents such as methylene chloride and THF, but the enzymes and their carriers cannot, which facilitates filtration for purification. The enzymes filtered from the product can be recycled so that it is an eco-friendly, economical and effective method for the condensation polymerization.

Condensation of lactic and glycolic oligomers can also be promoted through the use of ester coupling reagents. The advantage of this approach is that it avoids the need for higher temperature conditions which can promote both depolymerization and transesterification. For example, coupling reagents such as 1,1'-carbonyldiimidazol (CDI) and *N,N'*-dicyclohexyl-carboiimide (DCC) were introduced to facilitate the esterification of lactic acid and glycolic acid by Akutsu.<sup>9</sup> Byproducts such as *N,N'*-dicyclohexylurea were easily removed because of their insolubility in most organic solvents, and the molecular weight of polymer product (PLA) reached 15,800 ( $M_n$ ) after 24 h reaction at RT. The reaction also exploited 4-dimethylamino pyridine (DMAP) as a Lewis base catalyst. Unfortunately, the nucleophilic amine on the pyridine can also attack the acyl, resulting in suppression of the polymerization.<sup>36</sup> This is the main drawback of DCC/DMAP coupling condensation polymerization. By using *p*-toluenesulfonic

acid to neutralize the amine on DMAP, Stupp *et al.*, avoided the suppression effect, and succeeded in making polyesters with high degree of polymerization under room temperature.<sup>37</sup>

### 1.3 COMPOSITION OF PLA AND PLGA (MICROSTRUCTURE ANALYSIS)



**Figure 4.** Stereochemistry in poly(lactic acid)

PLA and PLGA polymers, because of the existence of the carbon chiral center on the lactic moiety, exhibit stereochemistry in their microstructures. The stereochemical variations reported include atactic, isotactic, syndiotactic and hemi-isotactic (Figure 4). As stereochemistry plays an

important role in the determination of physical and mechanical properties of polymers, the control of microstructure has been extensively investigated.<sup>38-46</sup> For the synthesis of PLA, the generally used ring opening polymerization usually generates isotactic and atactic PLAs.<sup>46</sup> Spassky *et al.*, using (R)-(SalBinap)-AlOCH<sub>3</sub> as catalyst, studied the ring opening polymerization of *rac*-lactide, and found that the polymer prepared was predominated isotactic.<sup>43</sup> Ovitt *et al.*, using (R)-(SalBinap)AlO<sup>*i*</sup>Pr to catalyze ROP of *meso*-lactide, obtained optical pure syndiotactic D,L-PLA.<sup>42</sup>

In describing the microstructure of polymers with multiple possible stereosequences, a convention of labeling the diastereomeric relationships along the chain used. In atactic PLA, for example, the units with L- or D-lactic units near each other will generate both isotactic and syndiotactic units, where *i* is used to designate adjacent units with the same stereochemistry and *s* is the label for adjacent units with opposite stereochemistry. The notation *iii* indicates that all three units in a triad have the same absolute stereochemistry. Pure poly(L-lactic acid) or poly(D-lactic acid) have all *i* relationships along their chains. The notations *si* or *is* indicate that a syndiotactic unit connects to an isotactic unit or an isotactic unit connects to a syndiotactic unit in a triad.<sup>19,47</sup> If we consider a tetrad, which is a sequence of 4 stereoactive units, there will be eight possibilities including *iii*, *iis*, *isi*, *iss*, *ssi*, *sis*, *sii* and *sss*. Significant work has been reported on the use of <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy to identify the microstructure of PLAs.<sup>19,47</sup>

For PLGA, both the stereochemistry and the sequence of lactic acids and glycolic acids will affect the microstructure. From the previous discussion we can see that most ROP of a mixture of lactide and glycolide will give random PLGAs as will the direct condensation polymerization of lactic acid and glycolic acids.<sup>15,28</sup> Only the ROP of D,L-3-methylglycolide gives some control of sequence but the sequence control is not perfect and the difficulty in preparing the stereopure

3-methylglycolide means that the resulting polymer is atactic.<sup>14</sup> PLGA prepared by condensation polymerization of *o*-(2-bromopropionyl) glycolic acid has excellent alternating structures of monomer units.<sup>31</sup> However, the first description of the synthesis of PLGAs with a variety of sequences was published recently by our group.<sup>44,48</sup>

The characterization of microstructure of PLGA polymers is more complex than for PLA. Most published studies have focused only on the content and the sequence of lactic and glycolic units. Kasperczyk has studied the microstructure of random poly(L-lactic-*co*-glycolic acid) copolymers using <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy.<sup>40</sup> The <sup>1</sup>H NMR spectrum of the glycolic methylene protons, whose chemical shift can be affected by the both by the lactic and glycolic units nearby, was found to correlate to a certain extent with the monomer sequence in the copolymer. Hausberger *et al.*, studied the connection between lactic and glycolic units in the poly(D,L-lactic-*co*-glycolic acid) copolymer.<sup>49</sup> Using <sup>13</sup>C NMR spectroscopy, they reported that the chemical shift of carbonyl group on glycolic unit depends on the identity of the neighboring monomers. The glycolic-lactic connection increased the chemical shift more than the glycolic-glycolic connection (~0.08 ppm) and the integration of these two signals can be used to estimate the ratio of lactic/glycolic acids in the copolymer.

When considering the stereochemistry of lactic units in PLGA, the question becomes even more complex. For alternating PLGA, if the PLA notation is employed, the *ii* in PLGA means two units with the same stereochemistry separated by a glycolic unit. Gao *et al.*, who has analyzed the microstructure of PLGA by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, roughly defined the tetrads signals in the spectra, and also showed the way to confirm the ratio of lactic/glycolic acid units in PLGA random copolymer.<sup>39</sup> In this system, however, the signals of some tetrads are still ambiguous. In our group, Ryan Stayshich synthesized poly (D,L-lactic-*alt*-glycolic acid) and

studied the microstructure of the polymer by the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy.<sup>44</sup> Signals for six of eight possible tetrads of methylene groups of glycolic units were clearly shown in the NMR spectrum. Signals for the remaining tetrads can be inferred. This result offers a more complete assignment for the methylene resonances in alternating PLGA.

The reason why the microstructure of PLA and PLGA has attracted such interest is that sequence determines, in part, the properties of polymers. The temperature of glass transition ( $T_g$ ) and the melting point ( $T_m$ ) are, for example, key thermophysical properties of polymers that depend not only on weight, thermal history and purity of polymers<sup>50,51</sup> but also on the monomer sequence and tacticity of the material. For isotactic PLA, the  $T_g$  is about 55 °C and  $T_m$  is 170-180 °C.<sup>52</sup> Since crystallinity depends on the packing of the isotactic units, the highest  $T_m$  requires optical purity of 72%-75%, which means the chain has at least 30 isotactic lactic units.<sup>53</sup> At lower optical purities, e.g. 47%, the  $T_m$  can decrease to 99 °C because the degree of crystallinity decreases.<sup>54</sup> A pure syndiotactic PLA which was prepared by Ovitt exhibits a  $T_m$  at 152 °C.<sup>55</sup> A possible explanation for the lower  $T_m$  compared with isotactic PLA is the presence of defects in the polymer during the polymer synthesis.<sup>52</sup> The stereocomplex of PLLA and PDLA, formed by co-crystallization, usually exhibits a higher  $T_m$  than isotactic PLA. When Spassky used a stereoselective catalyst to synthesize PLLA from D,L-lactide, a polymer with  $T_m$ =187 °C was obtained.<sup>43</sup> The  $T_m$  of this polymer is higher than that of isotactic PLA because the polymer chain is composed of both PLLA and PDLA sequences and these two sequences form a stereocomplex that crystallizes. This kind of PLLA and PDLA stereocomplexation, after optimizing the mixing ratio, can result in a  $T_m$  of 230 °C,<sup>56,57</sup> which is 50 °C higher than isotactic PLA, and it has been further studied because of this observation.<sup>42,45,58</sup>



Stereocomplexation of PLGA has also attracted significant study.<sup>59</sup> Random PLGA is noncrystallizable, and no  $T_m$  is observed by DSC. However, if the PLLA (PDLA) homopolymer is added to the PDLGA (PLLGA) copolymer to form the polymer blends, the material will crystallize. In blends of the two copolymers PLLGA and PDLGA, if the percentage of glycolic units decreases in each polymer, the  $T_m$  of blend will also increase.<sup>56,57</sup> Poly(glycolic acid) (PGA) itself will also form a homocrystals. Although crystal formation is more facile for PGA,<sup>60</sup> the value of long period and lamellar thickness of PGA crystalline is lower than those of PDLA and PLLA homocrystals,<sup>60</sup> and the glycolic units in the PLGA not disturb the homocrystallization between PLGA and PLA. This combination of factors results in a lower  $T_m$  with the increase of glycolic units in the blend, and offers a way to control the thermal properties of polymers.

Being studied as widely used biodegradable materials, the degradation rate has also related to the composition of polymers. The  $T_g$  of PLA is about 57 °C<sup>61</sup> and that of PLGA is above 37 °C,<sup>5</sup> which means under the physiological temperature, both polymers have rigid chain structure and sufficient mechanical strength to be drug carriers above a certain molecular weight.<sup>50</sup> During the hydrolysis process, ester bonds in the linear aliphatic chain are cleaved. Due to steric hindrance, however, the bonds between lactic units exhibit a slow cleavage rate than lactic-glycolic bonds which are in turn slower than glycolic-glycolic bonds.<sup>61,62</sup> Although D,L-PLA copolymer, which has more irregularities than the L-PLA, is employed more often because the irregularities result in a more amorphous in the polymer matrix and enable more homogeneous dispersion of loaded drugs,<sup>63</sup> the homopolymer actually degrades much slower than the *rac*-polymer because the crystalline regions exhibit a much lower water uptake rate than amorphous regions.<sup>52</sup> Since hydrolytic rates of different ester bonds are different, the control of ratio of

lactic to glycolic units in the PLGA copolymer offers an effective way to adjust the hydrolytic rate of the material.<sup>64</sup> PLGA with low content of glycolic units ( $\leq 10\%$ ) is nearly as slow to degrade as PLA. A glycolic content of 30% to 50% allows for tuning of the degradation rate. Increase in glycolic content beyond 50% is not typically useful as the hydrolytic rate of the glycolic blocks is simply too fast. Crystalline PGA with a 60,000  $M_w$  will degrade to near 3000  $M_w$  in two weeks.<sup>65</sup> As a result, PLGA with 50% glycolic units or less are usually chosen for biodegradation.<sup>66,67</sup> If the PLGA copolymers are processed to have a semi-crystalline structure, the hydrolytic rate will be slower than amorphous PLGA in the first week, because the semi-crystalline region will slow down the water uptake.<sup>65</sup> The rates will become the same after two weeks during which the glycolic semi-crystalline region has finished degradation and introduced water into the polymer matrix.

## 1.4 SIDE CHAIN MODIFICATION

Side chain modification can bring tunable properties for PLGA copolymers, such as an increase in hydrophilicity, the ability to attach new side chains, and the facile tuning of physical properties.<sup>16,68,69</sup> For example, bovine serum albumin (BSA) encapsulation efficiency can be improved by the presence of a pendent of hydroxymethyl group on PLGA.<sup>70</sup> BSA release profiles of thus prepared microparticles further regulated by tuning copolymer composition. Adhesion of stem cells to the polymer scaffold can also be improved by the introduction of functional side chains that increase hydrophilicity.<sup>71</sup> Side-chain functionality can also be used to improve the binding and delivery of DNA.<sup>72</sup>

Many studies have focused on the incorporation of reactive groups on PLGA copolymers by side chain modification,<sup>73,74</sup> using ROP of functionalized cyclic diester.<sup>16</sup> However, modification completed through the ROP of these lactide and glycolide derivatives will give a random distribution of the functional groups which is not ideal for all applications. The use of a sequenced copolymer, in contrast, would make it possible to distribute the pendant groups uniformly.

## **2.0 SYNTHESIS OF REPEATING SEQUENCED PLGA COPOLYMERS**

### **2.1 OVERVIEW**

In this chapter, are described the preparation and characterization of a variety of sequenced polymers of PLGA. Also discussed are modifications of synthetic strategies and the characterization of sequence fidelity by MALDI-TOF. Portions of this chapter have been published previously.<sup>75,76</sup>

### **2.2 INTRODUCTION**

We use Segmer Assembly Polymerization (SAP), an approach that entails the step-growth polymerization of exact sequenced segmers, to prepare sequenced PLGAs. The term “segmers” rather than oligomers or macromonomers is used to emphasize the fact that they are monodisperse units that bear end-groups which allow for polymerization. Using a SAP strategy, it is possible to encode sequences of modest length—we routinely prepare segmers of 2-8 monomers—to produce Repeating Sequence Copolymers (RSCs). The PLGA RSCs discussed herein were produced by the convergent coupling of orthogonally protected lactic and glycolic acids, followed by a DIC-mediated condensation polymerization.

Although PLGA copolymers and the homopolymers of lactic acid (PLAs) are more commonly prepared by the ring-opening polymerization (ROP) of lactides and glycolides the sequence complexity is limited relative to the SAP approach. The ROP strategy is a subset of chain-polymerizations that can, under certain conditions, be “programmed” using catalyst design and monomer reactivities to give sequenced microstructures. Indeed, the elegant work by key researchers who have used this approach in preparing polyolefins with controlled tacticities,<sup>77</sup> serves as an important inspirations for our interest in probing the role of sequence to a greater depth. Fundamentally, however, the “programmed” approach is limited with few exceptions to the alternation of two monomers.<sup>15</sup> In PLA, for example, where there are two lactic stereoisomers, there have been many reports of ROP-prepared polymers with controlled tacticity.<sup>78</sup> However, sequenced PLGAs, with the exception of the simple alternating copolymer,<sup>14,31</sup> cannot be prepared using ROP because no catalytic system exists that can create complex patterns of three monomers.

Two other approaches, templated synthesis and monomer-by-monomer construction, can produce sequenced copolymers,<sup>79,80</sup> but neither offers the versatility of SAP which can be applied to a wide variety of monomers and can be scaled up. Nature, of course, uses the template strategy to synthesize biopolymers. Chemists have both exploited Nature’s mechanisms to selectively prepare sequenced materials that are both naturally occurring and new. To date, however, there are only a few examples of synthetic templates prepared de novo for non-biological monomers. Monomer-by-monomer synthesis, as is used by commercial peptide synthesizers, can also be used to prepare complex exact sequences but the applicability of this approach to materials is limited because the method does not scale up well and there are practical limitations on chain molecular weights.

Stayshich from our group developed and reported the SAP-based synthesis of a variety of dimeric and trimeric segmers and the polymers derived from those segmers.<sup>48,76,81</sup> Although there is some redundancy between what has already appeared in the literature and the results described below, a complete synthetic section is included because some of the syntheses reported herein were conducted by the author of this dissertation in parallel to the report of Stayshich and because it is difficult to describe the preparation of the novel tetramer- and hexamers-based materials which are unique to this dissertation without discussing the creation of the dimeric and trimeric building blocks.

## **2.3 EXPERIMENTAL**

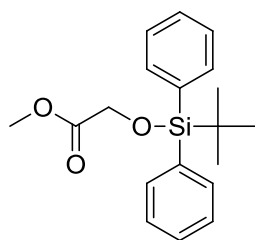
### **2.3.1 Materials and instrumentation**

Methyl glycolate, methyl lactate, glycolic acid, lactic acid, benzene, dimethylformamide, benzyl alcohol, benzyl bromide, and 1,8-diazabicycloundec-7-ene (DBU) were purchased from Acros and used as received. Dimethylaminopyridine, dicyclohexylcarbodiimide and *tert*-butyldiphenylchlorosilane were purchases from Oakwood Product Inc. and used as received. Diisopropylcarbodiimide, tetra-*n*-butylammonium fluoride (TBAF) was purchased from Aldrich. Lithium hydroxyl was purchased from Fisher. 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS) was synthesized according to the literature method.<sup>37</sup> Acetic acid (EMD) was purified by recrystallization. Ethyl acetate (Mallinckrodt), methylene chloride (EMD), and triethylamine (Acros) were distilled under nitrogen from calcium hydride. THF (Fisher, HPLC grade) was

passed through activated alumina using the SPS 400 (Innovative Technology). All other reagents were used as received without further purification.

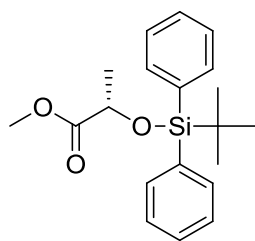
$^1\text{H}$  (300, 400 and 600 MHz) and  $^{13}\text{C}$  (75, 100 and 150 MHz) NMR spectra in  $\text{CDCl}_3$  were recorded with Bruker NMR spectrometers and calibrated to the residual solvent peaks ( $\delta$  7.24 and  $\delta$  77.0, respectively). Mass spectrometry for reagent characterization was conducted using Micromass Q-ToF ESI, Fisions VG autospec and Shimadzu LCMS-2020 instruments. Molecular weights and polydispersities of polymers were determined using a Waters SEC (THF) with Jordi 500 Å,  $10^3$  Å and  $10^4$  Å columns, a refractive index detector (Waters 2414), and polystyrene standards. Molecular weights and polydispersities in DMF were acquired on a Waters SEC with Polymer Standard Service 105 Å, 103 Å and 102 Å columns and refractive index detector (Waters 2410) using polystyrene standards as calibration. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-ToF-MS) analysis was performed on a Voyager-DE Pro with a 337 nm nitrogen laser and 20 kv accelerating voltage. For MALDI-ToF-MS sample preparation, the polymer was dissolved in THF (1 mg/ml) and mixed with *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB, in THF, 40 mg/ml) as the matrix and potassium trifluoroacetic acid (KTFA, in THF, 1 mg/ml) as the cationization agent. The spectra were recorded in the reflection mode. The MALDI-ToF-MS data analysis was performed using Data Explorer version 4.0.

### 2.3.2 Synthesis



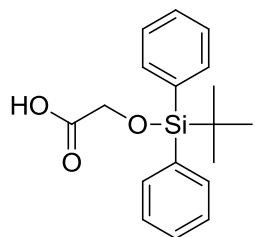
**Me-G-Si.** (JLi-2-1)*tert*-butyldiphenylchlorosilane (TBDPSiCl, 50.0 g, 182 mmol) was added to a solution of methyl 2-hydroxyacetate (Me-G, 15.0 g,

166 mmol), N, N-dimethylpyridin-4-amine (DMAP, 10.3 g, 84 mmol) and Et<sub>3</sub>N (37.3 g, 366 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (600 mL) in an ice bath. The mixture was stirred at RT for 20 h. The crude material was filtered to remove Et<sub>3</sub>NCl. The filtrate was washed with HCl (1M), brine and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated to give a pale yellow oil (54.5 g, 100% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.71-7.38 (m, 10H), 4.27 (s, 2H), 3.70 (s, 3H), 1.12 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI) m/z 328 (M<sup>+</sup>).



**Me-L-Si.** (JLi-5B-1) *Tert*-butyldiphenylchlorosilane (50.0 g, 182 mmol) was added to a solution of methyl 2-hydroxypropanoate (Me-L, 17.3 g, 166 mmol), N, N-dimethylpyridin-4-amine (10.3 g, 84 mmol) and triethylamine (Et<sub>3</sub>N, 37.3 g, 366 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (600 mL) in an ice bath.

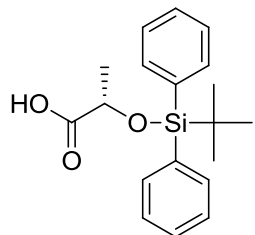
The mixture was stirred at RT for 20 h. The crude material was filtered to remove Et<sub>3</sub>NCl. The filtrate was washed with HCl (1M), brine and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated to give a clear oil (54.5 g, 100% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.71-7.37 (m, 10H), 4.31-4.29 (q, 1H, *J* = 6.6 Hz), 3.57 (s, 3H), 1.39-1.37 (d, 3H, *J* = 6.6 Hz), 1.10 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI) m/z 342 (M<sup>+</sup>).



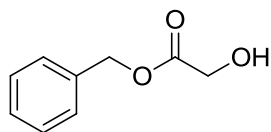
**G-Si.** (JLi-3B-1) LiOH H<sub>2</sub>O (7.4 g, 177 mmol dissolved in 300 mL H<sub>2</sub>O) was added dropwise to a solution of Me-G-Si (29 g, 88 mmol) in THF (400 mL) at 0 °C over 25 min. The mixture was stirred for 20 min and THF was removed under vacuum. The reaction mixture was diluted with 300 mL H<sub>2</sub>O and extracted with ether. The aqueous phase was acidified (pH < 3), extracted with ether, and the organic phase was dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated to give a clear oil (23.2 g, 83.6% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.70-7.40 (m, 10H), 4.28



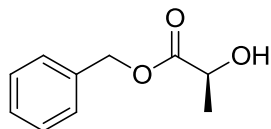
(s, 2H), 1.13 (s, 9H). MS (EI)  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> m/z 314 (M+).



**L-Si.** (JLi-10-1) LiOH  $\text{H}_2\text{O}$  (4.2 g, 100 mmol dissolved in 250 mL  $\text{H}_2\text{O}$ ) was added dropwise to a solution of Me-L-Si (17.1 g, 50 mmol) in THF (450 mL) at 0 °C over 25 min. The mixture was stirred for 5 h and THF was removed under vacuum. The reaction mixture was diluted with 135 mL  $\text{H}_2\text{O}$  and extracted with ether. The aqueous solution was acidified ( $\text{pH} < 3$ ), extracted with ether and the organic phase was dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated to give a white solid (12.5 g, 76% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.86-7.40 (m, 10H), 4.38-4.31 (q, 1H,  $J = 6.9$  Hz), 1.32-1.30 (d, 3H,  $J = 6.9$  Hz), 1.13 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> MS (EI) m/z 328 (M+).

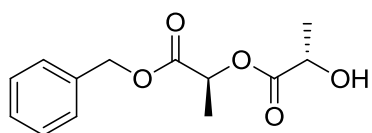


**Bn-G.** (JLi-16-1) Benzyl bromide (135 g, 789 mmol) was added dropwise to a solution of glycolic acid (50 g, 657 mmol) and DBU (100 g, 657 mmol) in benzene (1200 mL). The mixture was refluxed for 5 h and washed with HCl (1 M), water and brine. The crude material was dried with  $\text{MgSO}_4$ , filtered and concentrated. A clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 20% EtOAc in hexanes (75.3 g, 69% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.39 (s, 5H), 5.24 (s, 2H), 4.22-4.20 (d, 2H,  $J = 5.4$  Hz), 2.54-2.51 (q, 1H,  $J = 5.4$  Hz).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> MS (EI) m/z 166 (M+).

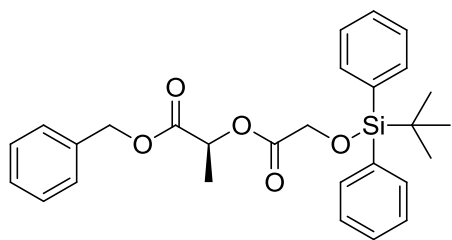


**Bn-L.** (JLii-5-2) DBU (436.1 g, 2.86 mol) was added dropwise to a solution of lactic acid (258 g, 2.86 mol) in methanol (350 mL) in an ice bath. The mixture was stirred for 30 min and distilled to light yellow (33 °C, 0.1 mmHg). After DMF (500 mL) was added, benzyl bromide (498 g, 2.86 mol) was added

dropwise. The solution was stirred at RT for 20 h and DMF was removed under vacuum (30 °C, 0.1 mmHg). The residue was dissolved in EtOAc and washed with water, HCl (1 M), NaHCO<sub>3</sub>, water and brine. The organic phase was dried with MgSO<sub>4</sub> and concentrated. A clear oil was isolated by distillation (80 °C, 10 mmHg, 344 g, 67% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.38 (s, 5H), 5.22 (s, 2H), 4.36-4.32 (q, 1H, *J* = 5.7 Hz), 2.99-2.98 (d, 1H, *J* = 5.4 Hz), 1.46-1.44 (d, 3H, *J* = 6.9 Hz). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI) *m/z* 180 (M<sup>+</sup>). MS (EI) *m/z* 180 (M<sup>+</sup>). **Bn-L<sub>rac</sub>** was prepared by the same procedure.

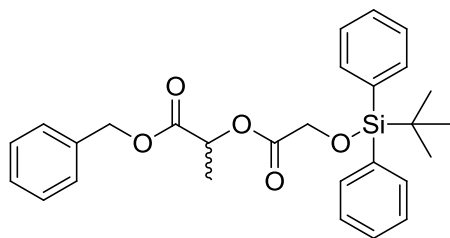


**Bn-LL.** (JLii-8-2) Lactide (4.3 g, 10 mmol) was added to a solution of N, N-dimethylpyridin-4-amine (3.7 g, 30 mmol) in benzyl alcohol (BnOH, 30 mL) at 40 °C. The mixture was stirred for 20 min and cooled down to RT. The crude material was filtered through silica gel to remove the DMAP. The residue was distilled (~60 °C, 30 mmHg) to remove the BnOH. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 10% EtOAc in hexanes, 4.9 g, 65% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.41-7.30 (m, 5H), 5.26-5.21 (q, 1H, *J* = 7.2 Hz), 5.23-5.19 (d, 1H, *J* = 12.0), 5.17-5.13 (d, 1H, *J* = 12.0), 4.39-4.31 (q, 1H, *J* = 6.9 Hz), 3.04-3.02 (d, 1H, *J* = 6.0), 1.55-1.53 (d, 3H, *J* = 7.2 Hz), 1.45-1.43 (d, 3H, *J* = 6.9 Hz). <sup>1</sup>H NMR is consistent with that reported previously.<sup>82-84</sup> MS (EI) *m/z* 252 (M<sup>+</sup>). MS (EI) *m/z* 252 (M<sup>+</sup>).



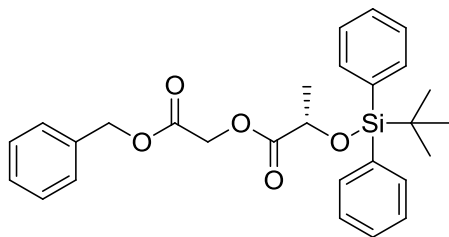
**Bn-LG-Si.** (JLiii-7-1) DCC (9.66 g, 46.6 mmol) was added to a solution of G-Si (14.64 g, 46.6 mmol), Bn-L (8.4 g, 46.6 mmol) and DMAP (2.84 g, 23.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (450 mL). The mixture was stirred for 4 h and filtered to remove the DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 2.5% EtOAc in hexanes, 15.1 g, 68% yield). <sup>1</sup>H NMR (300 MHz,

$\text{CDCl}_3$ )  $\delta$  7.69-7.32 (m, 15H), 5.23-5.16 (m, 3H), 4.41-4.35 (d, 1H,  $J = 13.5$  Hz), 4.34-4.28 (d, 1H,  $J = 13.5$  Hz), 1.48-1.46 (d, 3H,  $J = 7.2$  Hz), 1.11 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48,81</sup> MS (EI)  $m/z$  476 ( $M^+$ ).



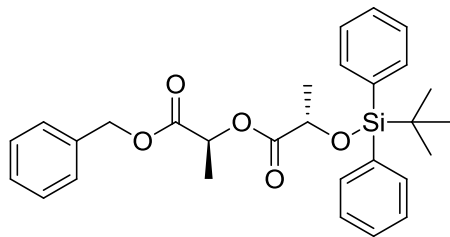
**Bn- $L_{rac}$ -G-Si.** (JLiii-4-2) DCC (10.1 g, 49 mmol) was added to a solution of Bn- $L_{rac}$  (8.8 g, 49 mmol), G-Si (15.3 g, 49 mmol), and DMAP (3 g, 24.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (500 mL). The mixture was stirred for 4 h and filtered to

remove the DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 2.5% EtOAc in hexanes, 9.5 g, 80% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70-7.33 (m, 15H), 5.21-5.14 (m, 3H), 4.40-4.34 (d, 1H,  $J = 16.5$  Hz), 4.33-4.27 (d, 1H,  $J = 16.8$  Hz), 1.47-1.45 (d, 3H,  $J = 7.2$  Hz), 1.10 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>81</sup> MS (EI)  $m/z$  476 ( $M^+$ ).



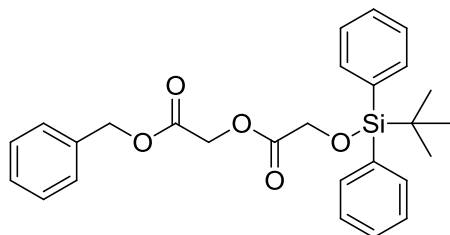
**Bn-GL-Si.** (JLiii-54-1) DCC (5.16 g, 25 mmol) was added to a solution of L-Si (8.2 g, 25 mmol), Bn-G (4.1 g, 25 mmol) and DMAP (1.5 g, 12.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (250 mL). The mixture was stirred for 4 h and filtered to

remove the DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 2.5% EtOAc in hexanes, 9.5 g, 80% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70-7.33 (m, 15H), 5.18 (s, 2H), 4.65-4.59 (d, 1H,  $J = 15.9$  Hz), 4.49-4.44 (d, 1H,  $J = 15.9$  Hz), 4.43-4.36 (q, 1H,  $J = 6.9$  Hz), 1.42-1.40 (d, 3H,  $J = 6.9$  Hz), 1.11 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> MS (EI)  $m/z$  476 ( $M^+$ ).



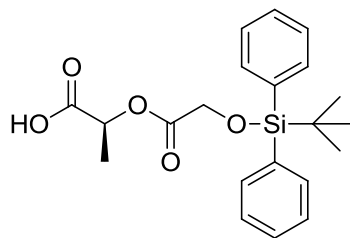
**Bn-LL-Si.** (JLiii-78-1) DCC (6.15 g, 29.8 mmol) was added to a solution of L-Si (9.79 g, 29.8 mmol), Bn-L (5.37 g, 29.8 mmol) and DMAP (1.82 g, 15 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (300 mL). The solution was stirred for 4 h and

filtered to remove the DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 2.5% EtOAc in hexanes, 9.3 g, 63.7% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.75-7.30 (m, 15H), 5.20-5.16 (d, 1H, *J* = 12.3 Hz), 5.13-5.09 (d, 1H, *J* = 12.3 Hz), 5.04-4.97 (q, 1H, *J* = 6.9 Hz), 4.37-4.30 (q, 1H, *J* = 6.9 Hz), 1.40-1.37 (d, 3H, *J* = 6.9 Hz), 1.35-1.33 (d, 3H, *J* = 7.2 Hz), 1.10 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI) *m/z* 490 (M<sup>+</sup>).



**Bn-GG-Si.** (JLiii-73-2) DCC (7.53 g, 36.5 mmol) was added to a solution of G-Si (11.47 g, 36.5 mmol), Bn-G (6.06 g, 36.5 mmol) and DMAP (2.24 g, 18.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (350 mL). the solution was stirred for 4 h and

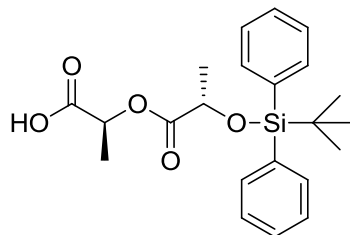
filtered to remove the DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 4% EtOAc in hexanes, 8.5 g, 50.4% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.71-7.34 (m, 15H), 5.21 (s, 2H), 4.69 (s, 2H), 4.39 (s, 2H), 1.12 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI) *m/z* 462 (M<sup>+</sup>).



**LG-Si.** (JLiii-74-1) Bn-LG-Si (14.3 g, 30 mmol) was dissolved in EtOAc (300 mL). Pd/C (0.35 g, 10%) was added to the solution under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged with nitrogen, filtered

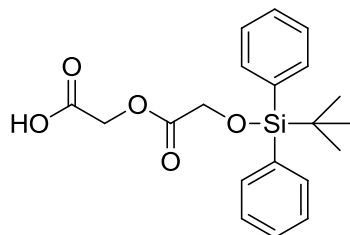
through celite and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15-25%

EtOAc in hexanes, 8.0 g, 69% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.71-7.37 (m, 10H), 5.19-5.12 (q, 1H,  $J = 7.2$  Hz), 4.42-4.36 (d, 1H,  $J = 16.8$  Hz), 4.35-4.30 (d, 1H,  $J = 16.8$  Hz), 1.52-1.50 (d, 3H,  $J = 7.2$  Hz), 1.11 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> HRMS ( $m+\text{Na}$ ) calc mass 409.1447, found 409.1419. HRMS calc mass 409.1447, found 409.1437.



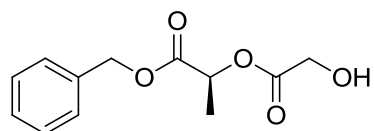
**LL-Si.** Bn-LL-Si (9.3 g, 19 mmol) was dissolved in EtOAc (200 mL). Pd/C (0.23 g, 10%) was added to the solution under nitrogen. The flask was purged and stirred overnight under hydrogen (1 atm). The mixture was purged with nitrogen, filtered

through celite and concentrated. A clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 15-25% EtOAc in hexanes, 4.7 g, 62% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.72-7.34 (m, 10H), 5.00-4.93 (q, 1H,  $J = 7.2$  Hz), 4.39-4.33 (q, 1H,  $J = 6.9$  Hz), 1.44-1.42 (d, 3H,  $J = 6.9$  Hz), 1.40-1.38 (d, 3H,  $J = 7.2$  Hz), 1.11 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> MS (EI)  $m/z$  400 ( $\text{M}^+$ ).

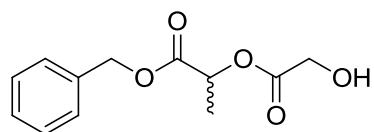


**GG-Si.** (JLii-18-1) Bn-GG-Si (12.9 g, 27.9 mmol) was dissolved in EtOAc (300mL). Pd/C (310 mg, 10%) was added to the solution under nitrogen. The flask was purged and stirred overnight under hydrogen (1 atm). The mixture was purged with

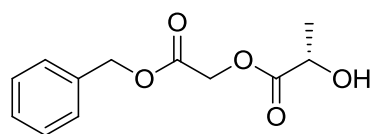
nitrogen, filtered through celite and concentrated. A white solid was isolated by flash chromatography ( $\text{SiO}_2$ , 15-25% EtOAc in hexanes, 7.2 g, 69.2 % yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  11.01 (s, 1H), 7.74-7.38 (m, 10H), 4.69 (s, 2H), 4.41 (s, 2H), 1.13 (s, 9H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> MS (EI)  $m/z$  372 ( $\text{M}^+$ ).



**Bn-LG.** (JLiii-78-2) TBAF (33 mL, 33 mmol) was added to a solution of Bn-LG-Si (10.5 g, 22 mmol) and HOAc (3.96 g, 66 mmol) in THF (220 mL) under nitrogen. The mixture was stirred for 4 h at RT and then washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-30% EtOAc in hexanes, 3.95 g, 75% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.42-7.31 (m, 5H), 5.29-5.22 (q, 1H, *J* = 7.2 Hz), 5.19 (s, 2H), 4.33-4.27 (d, 1H, *J* = 17.4 Hz), 4.26-4.20 (d, 1H, *J* = 17.4 Hz), 1.55-1.53 (d, 3H, *J* = 6.9 Hz). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 261.0739, found 261.0738.

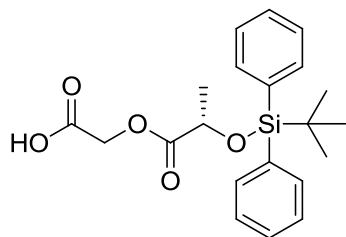


**Bn-L<sub>rac</sub>G** (JLiii-6-2) TBAF (29 mL, 29 mmol) was added to a solution of Bn-L<sub>rac</sub>G-Si (9.3 g, 20 mmol) and HOAc (4.7 g, 78 mmol) in THF (200 mL) under nitrogen. The mixture was stirred for 1 h at RT and then washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-30% EtOAc in hexanes, 3.95 g, 75% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.42-7.31 (m, 5H), 5.27-5.22 (q, 1H, *J* = 7.2 Hz), 5.19 (s, 2H), 4.30-4.18 (m, 2H), 1.53-1.51 (d, 3H, *J* = 7.2 Hz). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 261.0744, found 261.0738.



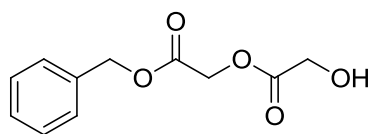
**Bn-GL.** (JLiii-80-1) TBAF (31 mL, 31 mmol) was added to a solution of Bn-GL-Si (9.8 g, 20.6 mmol) and HOAc (3.7 g, 62 mmol) in THF (300 mL) under nitrogen. The mixture was stirred for 4 h at RT and then washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil

was isolated by flash chromatography (SiO<sub>2</sub>, 15%-30% EtOAc in hexanes, 3.3 g, 67% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.41-7.34 (m, 5H), 5.21 (s, 2H), 4.83-4.78 (d, 1H, *J* = 15.9 Hz), 4.73-4.68 (d, 1H, *J* = 15.9 Hz), 4.45-4.38 (q, 1H, *J* = 6.9 Hz), 1.49-1.47 (d, 3H, *J* = 6.9 Hz). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 261.0739, found 261.0726.



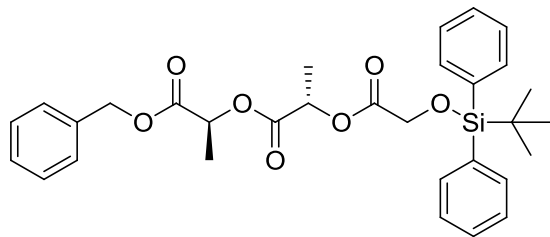
**GL-Si.** (JLiii-51-2) Bn-GL-Si (10.5 g, 22 mmol) was dissolved in EtOAc (220 mL). Pd/C (0.25 g, 10%) was added to the solution under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged with nitrogen, filtered

through celite and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15-25% EtOAc in hexanes, 5.5 g, 65% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.09 – 7.53 (m, 4H), 7.53 – 7.29 (m, 6H), 4.58 (d, *J* = 16.4 Hz, 1H), 4.47 (d, *J* = 16.4 Hz, 1H), 4.38 (q, *J* = 6.8 Hz, 1H), 1.40 (d, *J* = 6.8 Hz, 3H), 1.08 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 409.1447, found 409.1437.



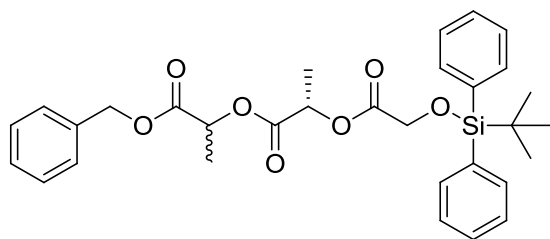
**Bn-GG.** (JLiii-75-1) TBAF (18 mL, 18 mmol, 1 M in THF), was added to a solution of Bn-GLG-Si (7.8 g, 14.6 mmol) and HOAc (3.4 g, 56.4 mmol) in THF (150 mL) under nitrogen.

The mixture was stirred for 30 min and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated to give a clear oil. White solid was obtained by recrystallization in hexanes (1.75 g, 77% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.40 – 7.25 (m, 5H), 5.18 (s, 2H), 4.73 (s, 2H), 4.28 (d, *J* = 5.9 Hz, 2H), 2.74 (t, *J* = 5.9 Hz, 1H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 247.0582, found 247.0573.



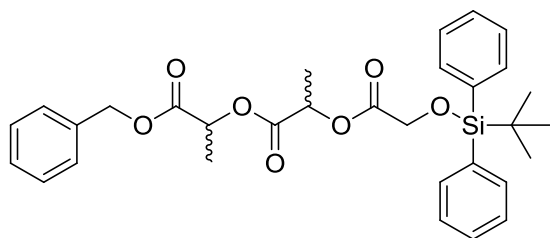
**Bn-LLG-Si.** (JLiii-8-1) DCC (3.0g, 14.2 mmol) was added to a solution of Bn-L (2.5 g, 14.2 mmol), LG-Si (5.5 g, 14.2 mmol) and DMAP (0.87 g, 7.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The mixture was

stirred for 2 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 6.4 g, 77% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.77 – 7.49 (m, 4H), 7.49 – 7.21 (m, 11H), 5.25 – 5.05 (m, 4H), 4.34 (d, *J* = 16.8 Hz, 1H), 4.26 (d, *J* = 16.8 Hz, 1H), 1.50 (d, *J* = 7.1 Hz, 3H), 1.43 (d, *J* = 7.1 Hz, 3H), 1.06 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 571.2128, found 571.2126.



**Bn-L<sub>rac</sub>LG-Si.** (JLiii-8-2) DCC (3.1g, 14.7 mmol) was added to a solution of Bn-L<sub>rac</sub> (2.6 g, 14.7 mmol), LG-Si (5.7 g, 14.7 mmol) and DMAP (0.9 g, 7.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The mixture was

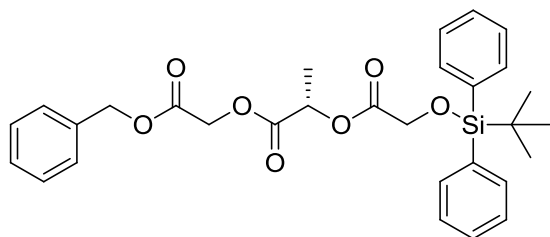
stirred for 2 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 6.7 g, 83% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.75 – 7.59 (m, 4H), 7.50 – 7.25 (m, 11H), 5.25 – 5.04 (m, 4H), 4.33 (d, *J* = 16.8 Hz, 1H), 4.27 (d, *J* = 16.6 Hz, 1H), 1.45 (d, *J* = 7.2 Hz, 3H), 1.43 (d, *J* = 7.2 Hz, 3H), 1.07 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 571.2128, found 571.2091.



**Bn-L<sub>rac</sub>L<sub>rac</sub>G-Si.** (JLiii-23-2) DCC (2.4g, 11.6 mmol) was added to a solution of Bn-L<sub>rac</sub> (2.1 g, 11.6 mmol), L<sub>rac</sub>G-Si (4.5 g, 11.6 mmol) and

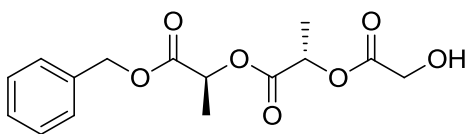


DMAP (0.7 g, 5.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (110 mL). The mixture was stirred for 2 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 4.9 g, 77% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.73 – 7.60 (m, 5H), 7.48 – 7.27 (m, 10H), 5.23 – 5.06 (m, 3H), 4.33 (d, *J* = 16.7 Hz, 1H), 4.26 (d, *J* = 16.4 Hz, 1H), 1.43 (m, 6H), 1.07 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 571.2128, found 571.2148.



**Bn-GLG-Si.** (JLiii-18-1) DCC (4.7 g, 22.6 mmol) was added to a solution of Bn-GL (5.4 g, 22.6 mmol), G-Si (7.1 g, 22.6 mmol) and DMAP (1.4 g, 11.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (250 mL). The

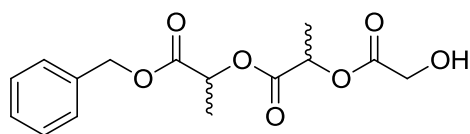
mixture was stirred for 2 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 8.4 g, 69% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.71-7.34 (m, 15H), 5.27-5.19 (m, 3H), 4.84-4.77 (d, *J* = 15.9 Hz, 1H), 4.59 (d, *J* = 15.9 Hz, 1H), 4.36 (d, *J* = 15.9 Hz, 1H), 4.28 (d, *J* = 15.9 Hz, 1H), 1.48-1.45 (d, *J* = 7.2 Hz, 3H), 1.10 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (*m*+Na) calc mass 557.1972, found 557.1922.



**Bn-LLG.** (JLiii-11-2) TBAF (18. mL, 18. mmol, 1 M in THF), was added to a solution of Bn-LLG-Si (6.4 g, 11.7 mmol) and HOAc (2.1 g, 35.0 mmol) in THF (125

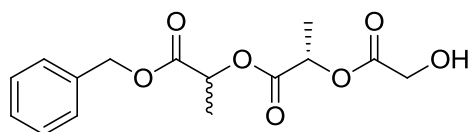
mL) under nitrogen. The mixture was stirred for 2 h and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 2.8 g, 78% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.43 – 6.99 (m, 5H), 5.28 –

4.96 (m, 4H), 4.27 (dd,  $J = 16.8, 4.7$  Hz, 1H), 4.20 (dd,  $J = 17.3, 5.6$  Hz, 1H), 2.37 (t,  $J = 5.6$  Hz, 1H), 1.52 (d,  $J = 7.1$  Hz, 3H), 1.51 (d,  $J = 7.1$  Hz, 3H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> HRMS (m+Na) calc mass 333.0950, found 333.0975.



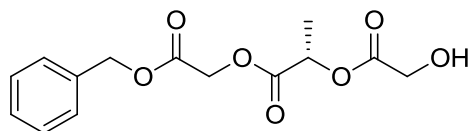
**Bn- $L_{rac}L_{rac}G$ .** (JLiii-32-2) TBAF (11 mL, 11 mmol, 1 M in THF), was added to a solution of Bn-

$L_{rac}L_{rac}G$ -Si (4.8 g, 8.6 mmol) and HOAc (2.6 g, 43 mmol) in THF (80 mL) under nitrogen. The mixture was stirred for 2 h and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 2.0 g, 74% yield).  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.41 – 7.26 (m, 5H), 5.37 – 4.97 (m, 4H), 4.31 – 4.13 (m, 2H), 2.42 – 2.32 (m, 1H), 1.54 – 1.45 (m, 6H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> HRMS (m+Na) calc mass 333.0950, found 333.0953.

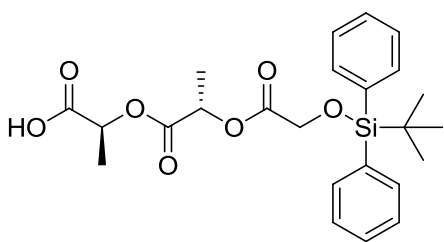


**Bn- $L_{rac}LG$ .** (JLiii-12-1) TBAF (18.3 mL, 18.3 mmol, 1 M in THF), was added to a solution of Bn-

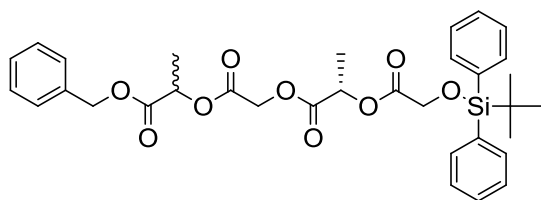
$L_{rac}LG$ -Si (6.7 g, 12.2 mmol) and HOAc (2.2 g, 36.7 mmol) in THF (125 mL) under nitrogen. The mixture was stirred for 2 h and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 3.4 g, 90% yield).  $^1\text{H}$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.43 – 7.26 (m, 5H), 5.36 – 5.05 (m, 4H), 4.23 (dd,  $J = 8.8, 3.4$  Hz, 2H), 2.38 – 2.25 (m, 1H), 1.52 (d,  $J = 6.9$  Hz, 3H), 1.49 (d,  $J = 6.9$  Hz, 3H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> HRMS (m+Na) calc mass 333.0950, found 333.0967.



**Bn-GLG.** (JLiii-18-2) TBAF (18 mL, 18 mmol, 1 M in THF), was added to a solution of Bn-GLG-Si (7.8 g, 14.6 mmol) and HOAc (3.4 g, 56.4 mmol) in THF (150 mL) under nitrogen. The mixture was stirred for 30 min and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 3.5 g, 81% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.42 – 7.24 (m, 5H), 5.26 (q, *J* = 7.1 Hz, 1H), 5.16 (s, 2H), 4.78 (d, *J* = 15.9 Hz, 1H), 4.61 (d, *J* = 15.9 Hz, 1H), 4.30 – 4.23 (d, *J* = 16.3 Hz, 1H), 4.20 (d, *J* = 16.3 Hz, 1H), 2.62 (s, 1H), 1.54 (d, *J* = 7.2 Hz, 3H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (m+Na) calc mass 319.0794, found 319.0804.

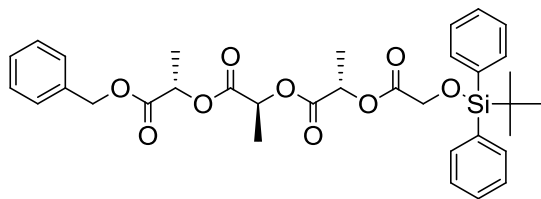


**LLG-Si.** (JLiii-65-1) Bn-LLG-Si (10.2 g, 18.6 mmol) was dissolved in EtOAc (220 mL). Pd/C (0.51 g, 10%) was added to the solution under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged with nitrogen, filtered through celite and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15-25% EtOAc in hexanes, 5.0 g, 59% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.78 – 7.57 (m, 4H), 7.48 – 7.32 (m, 6H), 5.22 – 5.14 (q, *J* = 7.1 Hz, 1H), 5.15 – 5.09 (q, *J* = 7.1 Hz, 1H), 4.34 (d, *J* = 16.8 Hz, 1H), 4.28 (d, *J* = 16.8 Hz, 1H), 1.53 (d, *J* = 7.1 Hz, 3H), 1.48 (d, *J* = 7.1 Hz, 3H), 1.06 (s, 9H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS (m+Na) calc mass 481.1658, found 481.1632.



**Bn-L<sub>rac</sub>GLG-Si.** (JLii-9B-1) DCC (3.42 g, 16.6 mmol) was added to a solution of Bn-L<sub>rac</sub>G (3.95 g, 16.6 mmol), LG-Si (6.4 g, 16.6 mmol) and

DMAP (1.0 g, 8.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The mixture was stirred for 3.5 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 8 g, 80% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.70-7.32 (m, 15H), 5.22-5.14 (m, 4H), 4.86-4.78 (d, 1H), 4.68-4.60 (d, 1H), 4.38-4.35 (d, 1H, *J* = 16.8 Hz), 4.32-4.29 (d, 1H, *J* = 16.8 Hz), 1.52-1.51 (d, 3H, *J* = 7.2 Hz), 1.51-1.50 (d, 3H, *J* = 7.2 Hz), 1.51-1.50 (d, 3H, *J* = 7.2 Hz). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 170.51, 170.50, 169.79, 169.78, 169.72, 169.69, 166.56, 166.51, 135.54, 135.51, 135.07, 132.64, 129.87, 128.58, 128.44, 128.14, 128.12, 127.77, 127.75, 69.42, 68.45, 68.39, 67.18, 61.91, 60.69, 60.68, 26.58, 19.21, 16.81, 16.78, 16.76, 16.74. MS (EI) *m/z* 606 (M<sup>+</sup>).

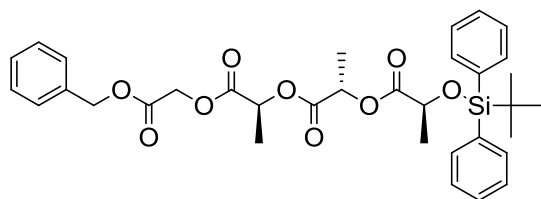


**Bn-LLL-Si.** (JLii-11B-1) DCC (2.98 g,

14.5 mmol) was added to a solution of Bn-LL (3.66 g, 14.5 mmol), LG-Si (5.6 g, 14.5 mmol) and

DMAP (0.89 g, 7.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The

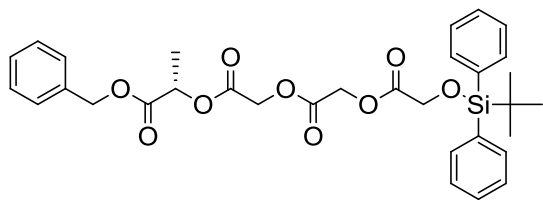
mixture was stirred for 3.5 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 6.7 g, 75% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.70-7.31 (m, 15H), 5.20-5.12 (m, 5H), 4.38-4.35 (d, 1H, *J* = 16.8 Hz), 4.32-4.29 (d, 1H, *J* = 16.8 Hz), 1.53-1.52 (d, 3H, *J* = 7.2 Hz), 1.52-1.51 (d, 3H, *J* = 7.2 Hz), 1.52-1.51 (d, 3H, *J* = 7.2 Hz), 1.08 (s, 9H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 170.56, 169.87, 169.85, 169.61, 135.52, 135.49, 135.04, 132.67, 132.63, 129.85, 128.56, 128.45, 128.19, 127.77, 127.75, 69.18, 68.85, 68.38, 67.13, 61.90, 26.57, 19.21, 16.70, 16.68, 16.52. MS (EI) *m/z* 620 (M<sup>+</sup>).



**Bn-GLLL-Si.** (JLi-35B-1) DCC (2.4 g, 11.7

mmol) was added to a solution of Bn-GL (2.8 g,

11.7 mmol), LL-Si (4.7 g, 11.7 mmol) and DMAP (0.7 g, 5.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The mixture was stirred for 3.5 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 4.9 g, 67% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 7.69-7.36 (m, 15H), 5.25-5.18 (q, 1H, *J* = 7.2 Hz), 5.19 (s, 2H), 5.01-4.94 (q, 1H, *J* = 6.9 Hz), 4.83-4.77 (d, 1H, *J* = 15.9 Hz), 4.62-4.57 (d, 1H, *J* = 15.9 Hz), 4.38-4.32 (q, 1H, *J* = 6.6 Hz), 1.56-1.54 (d, 3H, *J* = 6.9 Hz), 1.44-1.42 (d, 3H, *J* = 6.9 Hz), 1.41-1.39 (d, 3H, *J* = 7.2 Hz), 1.11 (s, 9H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 173.09, 169.86, 169.67, 166.90, 135.95, 135.74, 134.86, 133.44, 133.08, 129.77, 128.64, 128.45, 127.64, 127.56, 68.77, 68.49, 68.22, 67.25, 61.03, 26.78, 21.11, 19.20, 16.68, 16.52. MS (EI) *m/z* 620 (M<sup>+</sup>).

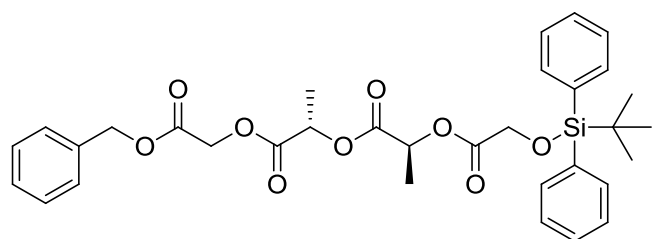


**Bn-LGGG-Si.** (JLii-18B-1) DCC (4.0 g,

19.3 mmol) was added to a solution of Bn-LG (4.6 g, 19.3 mmol), GG-Si (7.2 g, 19.3 mmol) and

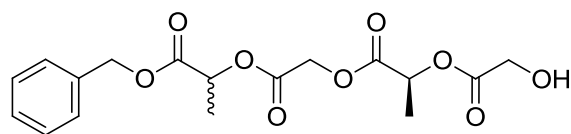
DMAP (1.2 g, 9.6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL). The

mixture was stirred for 1 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 8.3 g, 72% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 7.71-7.34 (m, 15H), 5.27-5.19 (m, 3H), 4.84-4.70 (m, 4H), 4.38 (s, 2H), 1.55-1.52 (d, 3H, *J* = 6.9 Hz), 1.10 (s, 9H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 170.45, 169.70, 166.78, 166.42, 135.49, 135.04, 132.54, 129.88, 128.57, 128.43, 128.11, 127.77, 69.48, 67.17, 61.77, 60.75, 60.30, 60.20, 26.55, 19.18, 16.71. MS (EI) *m/z* 592 (M<sup>+</sup>).



**Bn-GLLG-Si.** (JLiii-66-1) DCC (2.5 g, 10.7 mmol) was added to a solution of Bn-G (1.8 g, 10.7 mmol), LLG-Si (4.9 g, 10.8 mmol) and DMAP (0.65 g, 5.4 mmol)

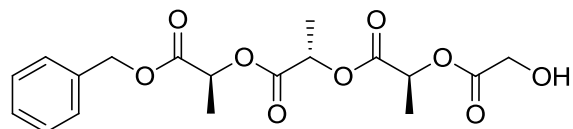
in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The mixture was stirred for 2 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 5% EtOAc in hexanes, 5.1 g, 79% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.71-7.34 (m, 15H), 5.27-5.20 (m, 2H), 5.16 (s, 2H), 4.77 (d, *J* = 15.9 Hz, 1H), 4.60 (d, *J* = 15.9 Hz, 1H), 4.27 (d, *J* = 15.9 Hz, 1H), 4.25 (d, *J* = 15.9 Hz, 1H), 1.55 (d, *J* = 7.1 Hz, 3H), 1.53 (d, *J* = 7.1 Hz, 3H), 1.10 (s, 9H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 170.73, 170.13, 169.69, 167.11, 135.79, 135.33, 132.90, 130.15, 128.85, 128.74, 128.46, 128.05, 69.53, 69.34, 67.43, 62.09, 60.61, 26.86, 19.48, 16.98, 16.85. HRMS (*m*+Na) calc mass 629.2183, found 629.2201.



**Bn-L<sub>rac</sub>GLG.** (JLii-24B-1) TBAF (20 mL, 20 mmol, 1 M in THF) was added to a solution of Bn-L<sub>rac</sub>GLG-Si (8 g, 13.2 mmol) and HOAc (2.38

g, 40 mmol) in THF (130 mL) under nitrogen. The mixture was stirred for 2 h and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 4 g, 82% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.38-7.33 (m, 5H), 5.32-5.16 (m, 4H), 4.89-4.82 (d, 1H), 4.72-4.65 (d, 1H), 4.31-4.28 (d, 1H, *J* = 17.4 Hz), 4.26-4.23 (d, 1H, *J* = 17.4 Hz), 1.61-1.59 (m, 3H), 1.54-1.52 (m, 3H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 172.36, 172.35, 169.65, 169.61, 169.52, 169.45, 166.39, 166.35,

134.95, 128.41, 128.27, 127.96, 127.95, 70.03, 69.33, 68.74, 68.71, 67.02, 60.67, 60.36, 60.25, 60.20, 16.60, 16.58, 16.55, 16.54. MS (EI)  $m/z$  368 ( $M^+$ ).

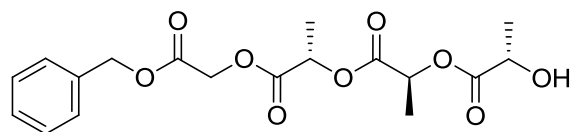


**Bn-LLLG.** (JLii-11B-2) TBAF (32 mL,

32 mmol, 1 M in THF) was added to a solution of

Bn-LLLG-Si (13.3 g, 21.5 mmol) and HOAc

(3.9 g, 64 mmol) in THF (210 mL) under nitrogen. The mixture was stirred for 2 h and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 6.2 g, 76% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.39-7.32 (m, 5H), 5.26-5.23 (q, 1H,  $J$  = 7.2 Hz), 5.22-5.5.18 (q, 1H,  $J$  = 7.2 Hz), 5.21-5.17 (q, 1H,  $J$  = 7.2 Hz), 5.21-5.19 (d, 1H,  $J$  = 12.6 Hz), 5.15-5.13 (d, 1H,  $J$  = 12.6 Hz), 4.32-4.28 (q, 1H), 4.26-4.23 (q, 1H), 1.61-1.60 (d, 3H,  $J$  = 7.2 Hz), 1.56-1.54 (d, 3H,  $J$  = 7.2 Hz), 1.54-1.53 (d, 3H,  $J$  = 7.2 Hz). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  173.14, 172.85, 172.03, 168.47, 134.76, 128.69, 128.53, 128.43, 128.19, 69.27, 69.15, 68.84, 67.34, 61.13, 16.98, 16.59, 16.21. MS (EI)  $m/z$  382 ( $M^+$ ).



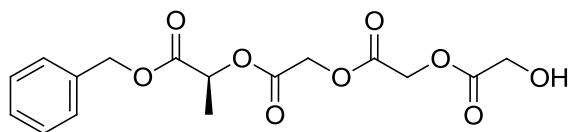
**Bn-GLLL.** (JLi-36-1) TBAF (9 mL, 9

mmol, 1 M in THF), was added to a solution of

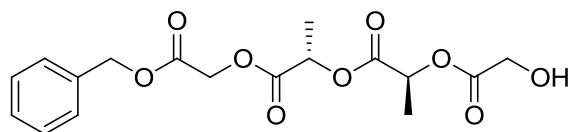
Bn-GLLL-Si (4.9 g, 7.9 mmol) and HOAc (1.22

g, 20 mmol) in THF (100 mL) under nitrogen. The mixture was stirred for 2 h and washed with brine. The brine was extracted with Et<sub>2</sub>O. Organic fractions were combined and dried with MgSO<sub>4</sub>. The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography (SiO<sub>2</sub>, 15%-35% EtOAc in hexanes, 1.8 g, 60% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.37-7.36 (m, 5H), 5.28-5.16 (m, 4H), 4.85-4.79 (d, 1H,  $J$  = 15.9 Hz), 4.64-4.59 (d,

1H,  $J = 15.9$  Hz), 4.40-4.33 (q, 1H,  $J = 6.9$  Hz), 1.61-1.57 (m, 6H), 1.51-1.49 (d, 3H,  $J = 6.9$  Hz).  $^{13}\text{H}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  172.55, 169.89, 169.52, 169.44, 169.30, 135.01, 128.60, 128.52, 128.24, 69.27, 69.15, 69.10, 67.20, 60.46, 16.80, 16.73, 16.59. MS (EI)  $m/z$  382 ( $\text{M}^+$ ).



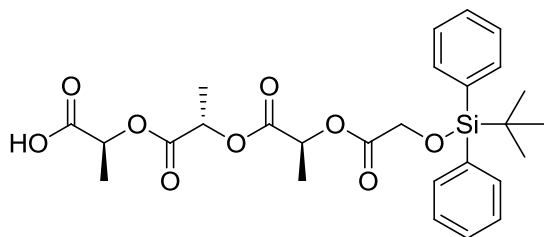
**Bn-LGGG.** TBAF (18 mL, 18 mmol, 1 M in THF), was added to a solution of Bn-LGGG-Si (9.8 g, 16.5 mmol) and HOAc (5.0 g, 83 mmol) in THF (150 mL) under nitrogen. The mixture was stirred for 40 min and washed with brine. The brine was extracted with  $\text{Et}_2\text{O}$ . Organic fractions were combined and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 15%-35% EtOAc in hexanes, 4.1 g, 70% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42-7.32 (m, 5H), 5.28-5.21 (q, 1H,  $J = 7.2$  Hz), 5.19 (s, 2H), 4.85 (s, 2H), 4.86-4.81 (d, 1H,  $J = 15.9$  Hz), 4.79-4.74 (d, 1H,  $J = 16.2$  Hz), 4.32 (s, 2H), 1.55-1.53 (d, 3H,  $J = 6.9$  Hz).  $^{13}\text{H}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  172.62, 169.85, 169.59, 168.95, 134.79, 128.87, 128.65, 128.43, 128.17, 69.27, 67.34, 61.94, 61.31, 60.58, 16.59. MS (EI)  $m/z$  354 ( $\text{M}^+$ ).



**Bn-GLLG.** (JLiii-66-2) TBAF (12.3 mL, 12.3 mmol, 1 M in THF), was added to a solution of Bn-GLLG-Si (5.1 g, 8.0 mmol) and HOAc (3.9 g, 65.0 mmol) in THF (80 mL) under nitrogen. The mixture was stirred for 30 min and washed with brine. The brine was extracted with  $\text{Et}_2\text{O}$ . Organic fractions were combined and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 15%-35% EtOAc in hexanes, 2.2 g, 71% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.39 – 7.29 (m, 5H), 5.24 (q,  $J = 7.1$  Hz, 1H), 5.20 (q,  $J = 7.1$  Hz, 1H), 5.17 (s, 2H), 4.78 (d,  $J = 15.9$  Hz, 1H), 4.60 (d,  $J = 15.9$  Hz, 1H), 4.28 (d,  $J = 17.3$  Hz, 1H), 4.21 (d,  $J = 17.5$  Hz, 1H), 2.31 (s, 1H), 1.57 (d,  $J = 7.1$  Hz, 3H),



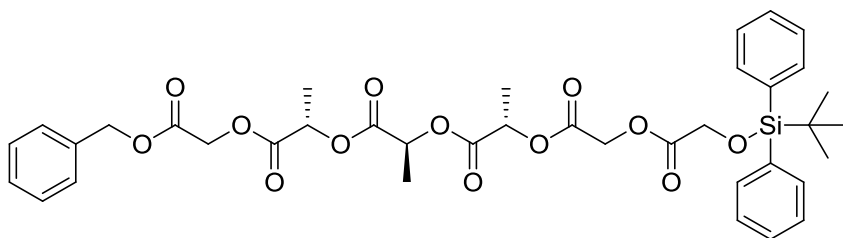
1.55 (d,  $J = 7.1$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.69, 169.58, 169.53, 166.89, 134.78, 128.64, 69.12, 69.02, 67.31, 61.09, 60.46, 16.69. HRMS ( $m+\text{Na}$ ) calc mass 391.1005, found 391.0982.



**LLLG-Si.** (JLiii-75-2) Bn-LLLG-Si (6.9 g,

11.1 mmol) was dissolved in EtOAc (110 mL). Pd/C (0.35 g, 10%) was added to the solution under nitrogen. The flask was purged and stirred overnight

under  $\text{H}_2$  (1 atm). The mixture was purged with nitrogen, filtered through celite and concentrated. A clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 15-35% EtOAc in hexanes, 3.9 g, 66% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.71 – 7.61 (m, 4H), 7.45 – 7.30 (m, 6H), 5.20 – 5.07 (m, 3H), 4.34 (d,  $J = 16.8$  Hz, 1H), 4.27 (d,  $J = 16.8$  Hz, 1H), 1.55 (d,  $J = 7.4$  Hz, 4H), 1.54 (d,  $J = 7.5$  Hz, 3H), 1.49 (d,  $J = 7.1$  Hz, 3H), 1.06 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.75, 170.72, 170.65, 169.82, 135.56, 135.52, 132.67, 129.91, 127.81, 127.78, 68.58, 68.41, 68.32, 61.92, 26.59, 19.24, 16.70, 16.66, 16.64. MS (EI)  $m/z$  530 ( $\text{M}^+$ )

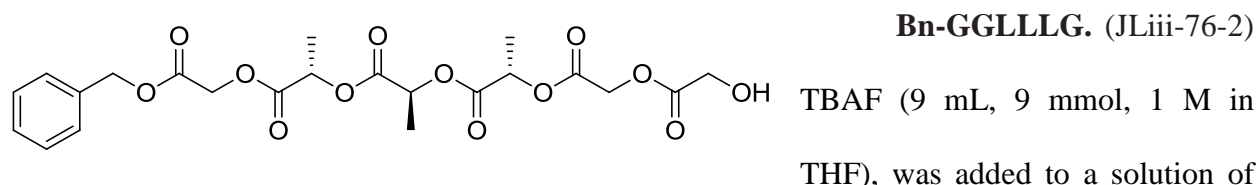


**Bn-GG-LLLG-Si.**

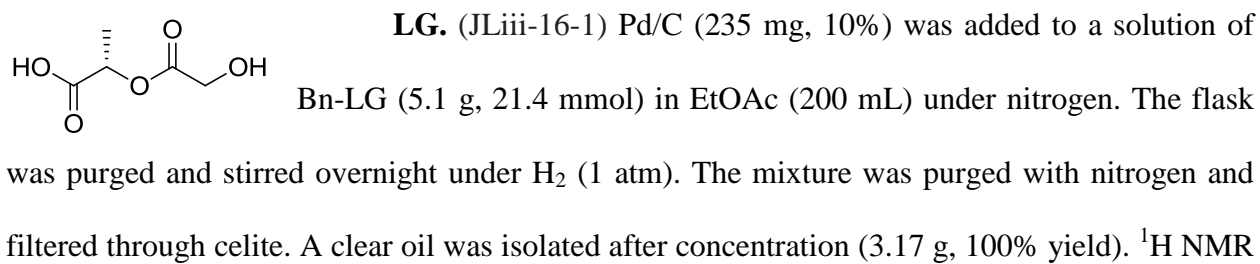
(JLiii-76-1) DCC (1.6 g, 7.4 mmol) was added to a solution of Bn-GG (1.7 g,

7.4 mmol), LLG-Si (3.9 g, 7.4 mmol) and DMAP (0.45 g, 3.7 mmol) in  $\text{CH}_2\text{Cl}_2$  (80 mL). The mixture was stirred for 2 h under RT and then filtered to remove DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 5% EtOAc in hexanes, 4.3 g, 80% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.67 (m, 4H), 7.48 – 7.29 (m, 11H), 4.86 (d,  $J = 16.1$  Hz, 1H), 5.19 (m, 5H), 4.73 (d,  $J = 15.9$  Hz, 1H), 4.70 (d,  $J = 15.9$  Hz, 1H), 4.68 (d,  $J =$

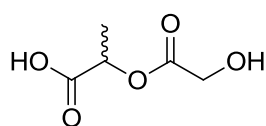
15.9 Hz, 1H), 4.66 (d,  $J = 15.9$  Hz, 1H), 4.35 (d,  $J = 16.8$  Hz, 1H), 4.28 (d,  $J = 16.8$  Hz, 1H), 1.61 – 1.57 (d,  $J = 7.1$  Hz, 3H), 1.59 – 1.55 (d,  $J = 7.1$  Hz, 3H), 1.50 (d,  $J = 7.1$  Hz, 3H), 1.07 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.62, 169.91, 169.66, 169.49, 166.79, 166.53, 135.56, 135.53, 134.81, 132.70, 132.67, 132.62, 129.88, 128.67, 128.65, 128.44, 127.80, 127.78, 68.89, 68.87, 68.41, 67.34, 61.92, 61.15, 60.67, 26.60, 19.24, 16.72, 16.69, 16.59. MS (EI)  $m/z$  736 (M+).



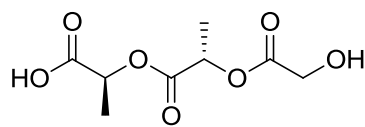
Bn-GGLLLG-Si (4.3 g, 5.8 mmol) and HOAc (2.1 g, 34.8 mmol) in THF (60 mL) under nitrogen. The mixture was stirred for 30 min and washed with brine. The brine was extracted with  $\text{Et}_2\text{O}$ . Organic fractions were combined and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 15%-35% EtOAc in hexanes, 2.3 g, 80% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43 – 7.26 (m, 5H), 5.33 – 5.13 (m, 5H), 4.86 (d,  $J = 16.1$  Hz, 1H), 4.73 (d,  $J = 15.9$  Hz, 1H), 4.71 (d,  $J = 15.9$  Hz, 1H), 4.70 (d,  $J = 15.9$  Hz, 1H), 4.35 – 4.15 (m, 2H), 2.31 (t,  $J = 5.7$  Hz, 1H), 1.58 (m, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.72, 169.64, 169.51, 169.46, 166.82, 166.54, 134.80, 128.68, 128.67, 128.49, 128.45, 69.17, 69.10, 68.97, 67.37, 61.17, 60.70, 60.48, 16.74, 16.70, 16.63. MS (EI)  $m/z$  498 (M+).



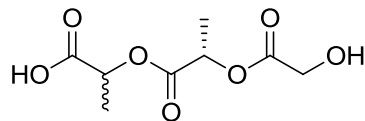
(300 MHz, CDCl<sub>3</sub>)  $\delta$  5.27-5.20 (q, 1H,  $J$  = 6.9 Hz), 4.35-4.30 (d, 1H,  $J$  = 17.4 Hz), 4.28-4.22 (d, 1H,  $J$  = 17.4 Hz), 1.58-1.56 (d, 3H,  $J$  = 6.9 Hz). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI)  $m/z$  148 (M<sup>+</sup>).



**L<sub>rac</sub>G** (JLiii-31-2) Pd/C (180 mg, 10%) was added to a solution of Bn-L<sub>rac</sub>G (3.9 g, 16.4 mmol) in EtOAc (200 mL) under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (2.0 g, 83% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.21 (q,  $J$  = 7.2 Hz, 1H), 4.30 (d,  $J$  = 17.4 Hz, 1H), 4.23 (d,  $J$  = 17.4 Hz, 1H), 1.55 (d,  $J$  = 7.2 Hz, 3H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> MS (EI)  $m/z$  148 (M<sup>+</sup>).



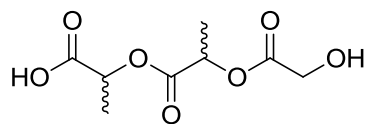
**LLG**. (JLiii-13-1) Pd/C (100 mg, 10%) was added to a solution of Bn-L<sub>rac</sub>LG (2.8 g, 9.0 mmol) in EtOAc (100 mL) under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (1.8 g, 92% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.20 (m, 2H), 4.30 (d,  $J$  = 17.4 Hz, 1H), 4.22 (d,  $J$  = 17.4 Hz, 1H), 1.57 (d,  $J$  = 7.1 Hz, 3H), 1.55 (d,  $J$  = 7.1 Hz, 3H). <sup>1</sup>H NMR is consistent with that reported previously.<sup>48</sup> HRMS ( $m$ +Na) calc mass 243.0481, found 243.0477.



**L<sub>rac</sub>LG**. (JLiii-13-2) Pd/C (120 mg, 10%) was added to a solution of Bn-L<sub>rac</sub>LG (3.4 g, 11.0 mmol) in EtOAc (110 mL) under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (2.2 g, 92% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.32 – 5.07 (m, 2H), 4.30 (d,  $J$  = 17.6 Hz, 1H),

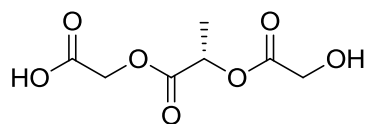
4.22 (d,  $J = 17.5$  Hz, 1H), 1.55 (m, 6H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup>

HRMS ( $m+\text{Na}$ ) calc mass 243.0481, found 243.0495.



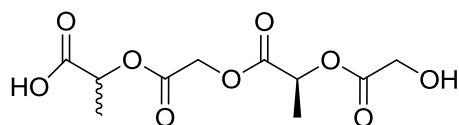
**$L_{rac}L_{rac}G$ .** (JLiii-33-1) Pd/C (80 mg, 10%) was added to a solution of Bn-GLG (2.0 g, 6.5 mmol) in EtOAc (60 mL) under

nitrogen. The flask was purged and stirred overnight under  $\text{H}_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (1.42 g, 95% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.43-4.91 (m, 2H), 4.40-3.88 (m, 2H), 1.57-1.50 (m, 6H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> HRMS ( $m+\text{Na}$ ) calc mass 243.0481, found 243.0488.



**GLG.** (JLiii-19-1) Pd/C (130 mg, 10%) was added to a solution of Bn-GLG (3.5 g, 11.8 mmol) in EtOAc (120 mL) under

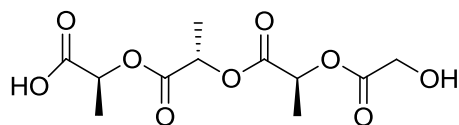
nitrogen. The flask was purged and stirred overnight under  $\text{H}_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (2.0 g, 82.3% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.27 (q,  $J = 7.1$  Hz, 1H), 4.76 (d,  $J = 16.3$  Hz, 1H), 4.64 (d,  $J = 16.3$  Hz, 1H), 4.30 (d,  $J = 16.3$  Hz, 1H), 4.24 (d,  $J = 16.3$  Hz, 1H), 1.56 (d,  $J = 7.1$  Hz, 3H).  $^1\text{H}$  NMR is consistent with that reported previously.<sup>48</sup> HRMS ( $m+\text{Na}$ ) calc mass 229.0324, found 229.0345.



**$L_{rac}GLG$ .** (JLii-24B-2) Pd/C (135 mg, 10%) was

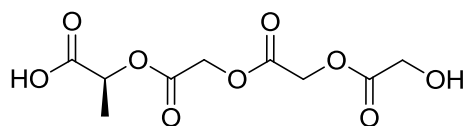
added to a solution of Bn- $L_{rac}GLG$  (4 g, 11 mmol) in EtOAc (100 mL) under nitrogen. The flask was purged and stirred overnight under  $\text{H}_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (3.17 g, 100% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.34-5.17 (m, 2H), 4.90-4.66 (m, 2H), 4.35-4.29 (d, 1H), 4.28-4.22 (d, 1H), 1.62-1.55 (m, 6H).  $^{13}\text{C}$  NMR (150 MHz)  $\delta$  174.57,

174.52, 172.89, 172.86, 171.73, 169.9, 169.85, 166.76, 166.72, 69.30, 69.29, 61.10, 60.74, 60.63, 21.23, 17.00, 16.99, 16.88, 14.36. MS (EI)  $m/z$  278 ( $M^+$ ).



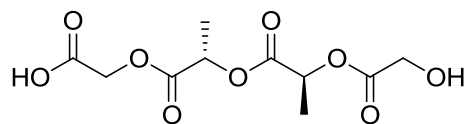
**LLL.G.** (JLii-12-1) Pd/C (178 mg, 10%) was added to a solution of Bn-LLL.G (6.2 g, 16.2 mmol) in EtOAc (150 mL) under nitrogen. The flask was purged and stirred

overnight under  $H_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (4.74 g, 100% yield).  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.26-5.23 (q, 1H,  $J = 7.2$  Hz), 5.23-5.19 (q, 1H,  $J = 7.2$  Hz), 5.19-5.15 (q, 1H,  $J = 7.2$  Hz), 4.32-4.29 (d, 1H,  $J = 16.8$  Hz), 4.26-4.24 (d, 1H,  $J = 17.4$  Hz), 1.61-1.59 (d, 3H,  $J = 6.6$  Hz), 1.60-1.59 (d, 3H,  $J = 6.6$  Hz), 1.57-1.56 (d, 3H,  $J = 6.6$  Hz).  $^{13}C$  NMR (150 MHz,  $CDCl_3$ )  $\delta$  174.65, 172.96, 171.66, 169.99, 169.77, 69.38, 69.10, 60.72, 60.67, 16.94, 16.86, 16.81. MS (EI)  $m/z$  292 ( $M^+$ ).



**LG.GG.** (JLii-23B-2) Pd/C (64 mg, 10%) was added to a solution of Bn-LGGG (2.0 g, 5.6 mmol) in EtOAc (50 mL) under nitrogen. The flask was purged and

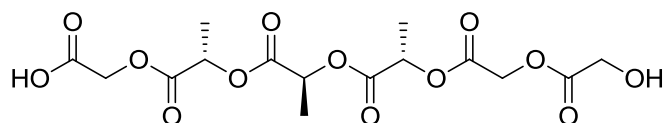
stirred overnight under  $H_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (1.49 g, 100% yield).  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.26-5.18 (q, 1H,  $J = 7.2$  Hz), 4.86-4.75 (m, 4H), 4.33 (s, 2H), 1.59-1.56 (d, 3H,  $J = 7.2$  Hz).  $^{13}C$  NMR (150 MHz,  $CDCl_3$ )  $\delta$  174.31, 172.84, 166.98, 166.67, 69.34, 61.18, 60.96, 60.62, 16.88. MS (EI)  $m/z$  264 ( $M^+$ ).



**G.LL.G.** (JLiii-67-2) Pd/C (110 mg, 10%) was added to a solution of Bn-GLLG (2.2 g, 6.0 mmol) in

EtOAc (60 mL) under nitrogen. The flask was purged and stirred overnight under  $H_2$  (1 atm).

The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (1.25 g, 75% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.30 – 5.18 (m, 2H), 4.77 (d,  $J$  = 16.4 Hz, 1H), 4.63 (d,  $J$  = 16.4 Hz, 1H), 4.29 (d,  $J$  = 17.4 Hz, 1H), 4.22 (d,  $J$  = 17.4 Hz, 1H), 1.58 (d,  $J$  = 7.1 Hz, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.75, 171.24, 169.75, 169.53, 69.16, 69.09, 60.78, 60.35, 16.63. HRMS (m+Na) calc mass 301.0536, found 301.0549.



**GGLLLG.** (JLiii-77-1) Pd/C (115

mg, 10%) was added to a solution of Bn-GGLLLG (2.3 g, 4.6 mmol) in EtOAc (50 mL) under nitrogen. The flask was purged and stirred overnight under  $\text{H}_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. A clear oil was isolated after concentration (1.8 g, 95% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.26 – 5.15 (m, 3H), 4.85 (d,  $J$  = 16.1 Hz, 1H), 4.72 (s, 2H), 4.69 (d,  $J$  = 16.1 Hz, 1H), 4.28 (d,  $J$  = 17.4 Hz, 1H), 4.22 (d,  $J$  = 17.4 Hz, 1H), 1.58 (d,  $J$  = 7.1 Hz, 3H), 1.58 (d,  $J$  = 7.1 Hz, 3H), 1.57 (d,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.75, 169.75, 169.53, 166.54, 69.20, 69.13, 69.02, 60.76, 60.72, 60.44, 16.72, 16.67, 16.62. MS (EI)  $m/z$  408 ( $\text{M}^+$ ).

**Poly LG.** (JLiii-17-1) DIC (0.7 g, 5.48 mmol) was added dropwise to a solution of LG (0.5 g, 2.74 mmol) and DPTS (0.16 g, 0.55 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.9 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (75 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (0.35 g, 70% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.30-5.22 (q, 1H,  $J$  = 7.2 Hz), 4.91-4.86 (d, 1H,  $J$  = 15.9 Hz), 4.68-4.63 (d, 1H,  $J$  = 15.9 Hz), 1.61-1.58 (d, 3H,  $J$  = 7.2 Hz).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.38, 166.44, 69.15, 60.82, 16.74. SEC (THF)  $M_n$  – 37.2 kDa; PDI: 1.40.

**Poly L<sub>rac</sub>G.** (JLiii-32-1) DIC (2.6 g, 20.1 mmol) was added dropwise to a solution of L<sub>rac</sub>G (2.0 g, 13.4 mmol) and DPTS (0.79 g, 2.7 mmol) in  $\text{CH}_2\text{Cl}_2$  (4.4 mL) in an ice bath. The

solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (300 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (1.4 g, 80% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.23 (m, 1H), 4.83 (m, 1H), 4.67 (m, 1H), 1.56 (m, 3H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.38, 169.31, 169.28, 169.22, 166.44, 166.40, 166.36, 69.19, 69.18, 69.16, 69.15, 60.82, 16.78, 16.76, 16.75, 16.74. SEC (THF)  $M_n$  – 30.0 kDa, PDI: 1.28.

**Poly LLG.** (JLiii-14-1) DIC (1.6 g, 12.4 mmol) was added dropwise to a solution of LLG (1.8 g, 8.3 mmol) and DPTS (0.49 g, 1.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (2.7 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (300 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (1.4 g, 84% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.19 (m, 2H), 4.85 (d,  $J$  = 16.0 Hz, 1H), 4.60 (d,  $J$  = 16.1 Hz, 1H), 1.57 (d,  $J$  = 7.2 Hz, 3H), 1.56 (d,  $J$  = 7.2 Hz, 3H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.48, 169.35, 166.48, 69.20, 69.00, 60.78, 16.69, 16.64. SEC (THF)  $M_n$  – 45.7 kDa, PDI: 1.50.

**Poly  $L_{rac}$ LG.** (JLiii-14-2) DIC (1.9 g, 14.9 mmol) was added dropwise to a solution of  $L_{rac}$ LG (2.20 g, 10.0 mmol) and DPTS (0.59 g, 2.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (3.3 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (300 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (1.9 g, 94% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.20 (m, 2H), 4.81 (m, 1H), 4.63 (m, 1H), 1.54 (m, 6H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.48, 169.45, 169.34, 169.29, 169.19, 169.17, 169.10, 166.46, 166.33, 69.36, 69.31, 69.18, 68.98, 60.78, 60.76, 16.71, 16.69, 16.67, 16.64. SEC (THF)  $M_n$  – 31.0 kDa, PDI: 1.46.

**Poly  $L_{rac}L_{rac}$ G,** (JLiii-33-2) DIC (0.8 g, 6.2 mmol) was added dropwise to a solution of  $L_{rac}L_{rac}$ G (0.95 g, 0.9 mmol) and DPTS (0.26 g, 1.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.4 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (100

mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (0.7 g, 80% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.21 (m, 2H), 4.81 (m, 1H), 4.64 (m, 1H), 1.55 (m, 6H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.51, 169.45, 169.36, 169.30, 169.27, 169.20, 169.15, 169.12, 166.52, 166.47, 166.35, 166.31, 69.34, 69.28, 69.16, 69.12, 69.05, 68.99, 68.97, 60.76, 16.72, 16.69, 16.63. SEC (THF)  $M_n$  – 30.0 kDa, PDI: 1.29.

**Poly GLG.** (JLiii-19-2) DIC (0.95 g, 7.5 mmol) was added dropwise to a solution of GLG (1.03 g, 5.0 mmol) and DPTS (0.29 g, 1.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.6 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (150 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (0.9 g, 96% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.25 (q,  $J = 7.1$  Hz, 1H), 4.86 (d,  $J = 16.1$  Hz, 1H), 4.80 (d,  $J = 16.1$  Hz, 1H), 4.72 (d,  $J = 16.1$  Hz, 1H), 4.68 (d,  $J = 16.1$  Hz, 1H), 1.57 (d,  $J = 7.1$  Hz, 3H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.27, 166.46, 166.35, 69.2, 60.87, 60.71, 16.72. SEC (THF)  $M_n$  – 24.3 kDa, PDI: 1.45.

**Poly  $L_{rac}$ GLG.** (JLii-14-1) DIC (0.7 g, 5.48 mmol) was added dropwise to a solution of  $L_{rac}$ GLG (0.5 g, 2.74 mmol) and DPTS (0.16 g, 0.55 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.9 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (75 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (0.35 g, 70% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.29-5.24 (m, 2H), 4.91-4.82 (m, 1H), 4.73-4.64 (m, 1H), 1.61-1.59 (m, 6H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.61, 169.46, 166.67, 166.63, 166.59, 69.43, 69.38, 61.05, 17.01, 16.97. SEC (THF)  $M_n$  – 12.4 kDa; PDI: 1.43.

**Poly LLLG.** (JLii-13B-1) DIC (0.5 g, 4 mmol) was added dropwise to a solution of LLLG (0.77 g, 2.6 mmol) and DPTS (0.12 g, 0.4 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (75 mL)



and stirred for 45 min. The precipitate was filtered and dried under nitrogen (0.38 g, 50% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.24-5.20 (q, 1H,  $J = 7.2$  Hz), 5.22-5.18 (q, 1H,  $J = 7.2$  Hz), 5.20-5.17 (q, 1H,  $J = 7.2$  Hz), 4.90-4.88 (d, 1H,  $J = 16.2$  Hz), 4.63-4.61 (d, 1H,  $J = 15.6$  Hz), 1.60-1.59 (d, 3H,  $J = 7.2$  Hz), 1.60-1.59 (d, 3H,  $J = 7.2$  Hz), 1.59-1.58 (d, 3H,  $J = 7.2$  Hz).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.75 (2x), 169.70, 166.74, 69.42, 69.32, 69.17, 61.01, 16.93, 16.89, 16.82. SEC (THF)  $M_n$  – 10.4 kDa; PDI: 1.80.

**Poly GLLL.** (JLii-4B-1) DIC (0.37 g, 3 mmol) was added dropwise to a solution of GLLL (0.57 g, 1.95 mmol) and DPTS (0.11 g, 0.39 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (75 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (0.34 g, 60% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.2-5.14 (m, 3H), 4.87-4.85 (d, 1H,  $J = 15.6$  Hz), 4.62-4.58 (d, 1H,  $J = 15.6$  Hz), 1.57-1.56 (m, 9H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.49, 169.43, 166.48, 69.16, 69.06, 68.91, 60.75, 16.67, 16.62, 16.56. SEC (THF)  $M_n$  – 8.7 kDa; PDI: 1.55.

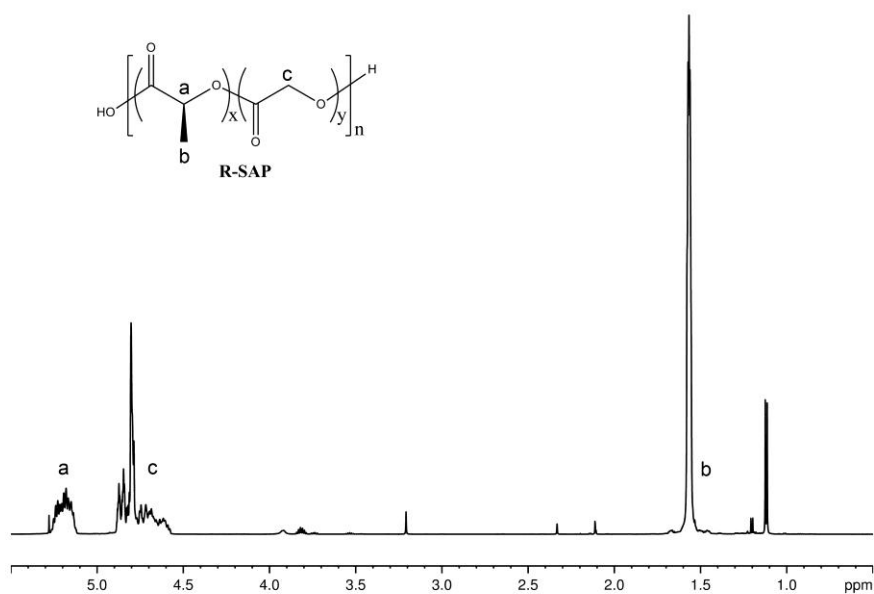
**Poly LGGG.** (JLii-24-2) DIC (1.1 g, 8.6 mmol) was added dropwise to a solution of LGGG (1.5 g, 5.7 mmol) and DPTS (0.33 g, 1.1 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.9 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (150 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (1.1 g, 73% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.29-5.26 (q, 1H,  $J = 7.2$  Hz), 4.90-4.72 (m, 6H), 1.60-1.59 (d, 3H,  $J = 7.2$  Hz),  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.48, 166.66, 166.61, 166.56, 69.47, 61.13, 60.99, 60.95, 16.92. SEC (THF)  $M_n$  – 7.8 kDa; PDI: 1.26.

**Poly GLLG.** (JLiii-68-1) DIC (0.85 g, 6.7 mmol) was added dropwise to a solution of GLLG (1.25 g, 4.5 mmol) and DPTS (0.27 g, 0.9 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.4 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (150 mL)

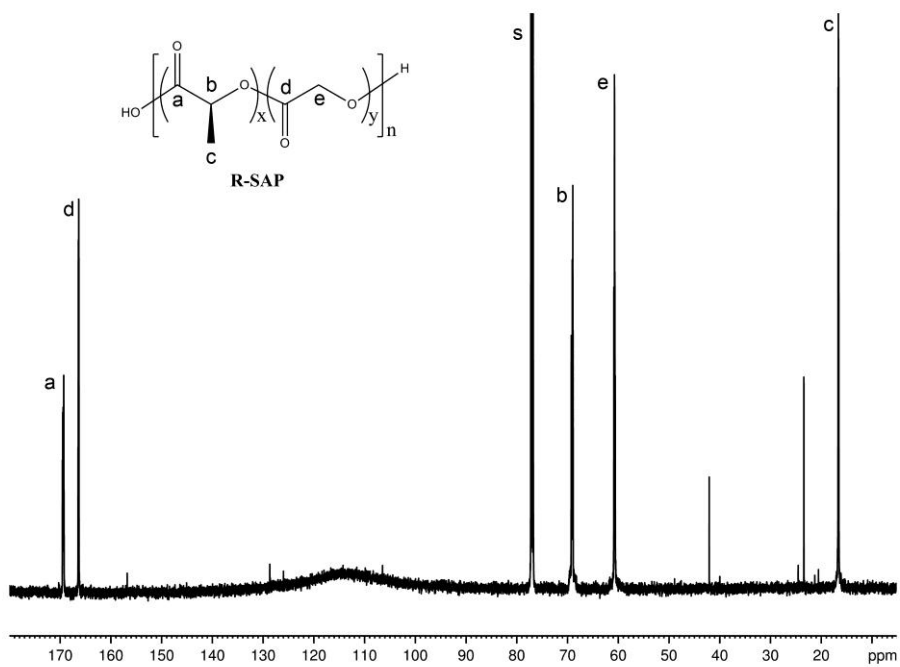
and stirred for 45 min. The precipitate was filtered and dried under nitrogen (1.1 g, 95% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.20 (m, 1H), 4.76 (m, 2H), 1.57 (m, 3H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.43, 169.33, 166.45, 166.43, 69.28, 69.00, 60.84, 60.65, 16.67, 16.62. SEC (THF)  $M_n$  – 35.0 kDa, PDI: 1.18.

**Poly GGLLLG.** (JLiii-77-2) DIC (0.8 g, 6.6 mmol) was added dropwise to a solution of GGLLLG (1.8 g, 4.4 mmol) and DPTS (0.26 g, 0.88 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.3 mL) in an ice bath. The solution was then stirred for 3 h under RT. The mixture was precipitated into methanol (150 mL) and stirred for 45 min. The precipitate was filtered and dried under nitrogen (1.55 g, 89% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.25 – 5.09 (m, 1H), 4.93 – 4.62 (m, 2H), 1.57 (d,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  169.50, 169.45, 169.41, 166.42, 166.40, 166.36, 69.29, 69.09, 68.94, 60.90, 60.72, 60.64, 16.68, 16.65, 16.58. SEC (THF)  $M_n$  – 45.9 kDa, PDI: 1.15.

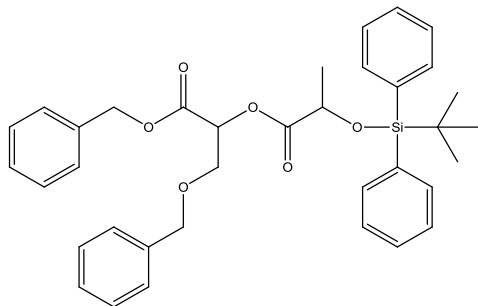
**R-SAP 50** (JLii-40B-1) was prepared as previously described.<sup>48</sup> Equivalent weights of each of the four segments LL, LG, GL and GG (0.34 g each, 2.26 mmol) were combined with (DPTS, 0.53 g, 1.8 mmol) and dissolved in  $\text{CH}_2\text{Cl}_2$ . After cooling the solution to 0 °C, 1,3 diisopropylcarbodiimide (DIC, 1.71 g, 13.6 mmol) was added dropwise and the solution was stirred at RT for 2 h. The product was precipitated from methanol (150 mL, 2x), filtered and dried under vacuum to give a white solid (1.1 g, 90% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ) see Figure 5 and Figure 6; SEC (THF)  $M_n$  – 27.4 kDa; PDI - 1.33.



**Figure 5.** <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) spectrum of R-SAP 50



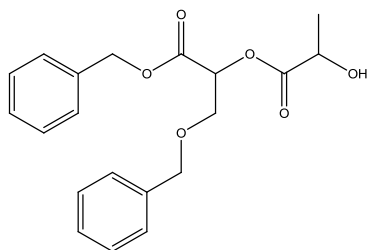
**Figure 6.** <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) spectrum of R-SAP 50



**Bn-S(Bn)L-Si.** (JLiii-35-1) DCC (2.9 g, 14 mmol)

was added to a solution of **Bn-S(Bn)** (4.0 g, 14 mmol), **L-Si** (4.6 g, 14 mmol) and DMAP (0.85 g, 7 mmol) in  $\text{CH}_2\text{Cl}_2$  (150 ml). The mixture was stirred for 4 h and filtered to remove the DCU. The filtrate was concentrated

and a clear oil was isolated by flash chromatography ( $\text{SiO}_2$ , 2.5% EtOAc in hexanes, 6.5 g, 78% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.74-7.20 (m, 20H), 5.20 (d, 1H,  $J = 12.4$  Hz), 5.15 (d, 1H,  $J = 12.0$  Hz), 5.16 (dd, 1H,  $J_1 = 4.8$  Hz,  $J_2 = 2.8$  Hz), 4.51 (d, 1H,  $J = 12.0$  Hz), 4.41 (d, 1H,  $J = 12.8$  Hz), 4.43 (q, 1H,  $J = 6.8$  Hz), 3.83 (dd, 1H,  $J_1 = 10.8$  Hz,  $J_2 = 4.8$  Hz), 3.57 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 3.2$  Hz), 1.42 (d, 3H,  $J = 6.8$  Hz). 1.10 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.31, 167.78, 137.65, 136.17, 135.98, 135.38, 135.32, 135.01, 133.63, 133.28, 130.01, 129.97, 129.87, 128.75, 128.59, 128.56, 128.47, 127.94, 127.87, 127.82, 127.79, 73.56, 72.26, 68.81, 68.74, 67.44, 27.00, 21.43, 19.43. HRMS ( $\text{M}+\text{Na}^+$ ) calc. 619.2492, found 619.2464.

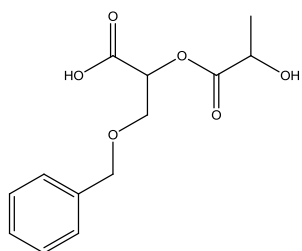


**Bn-S(Bn)L.** (JLiii-35-2) TBAF (5 ml, 5 mmol, 1M in

THF) was added to a solution of **Bn-S(Bn)L-Si** (2.3 g, 3.9 mmol) and acetic acid (0.4 ml, 7 mmol) in THF (40 ml). The mixture was stirred for 3 h and washed with  $\text{NaHCO}_3$  (40 ml saturated, 2x) and

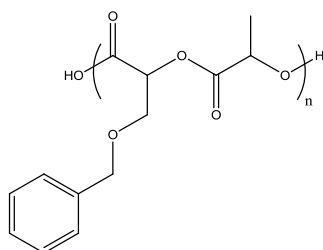
brine (1x). The aqueous solution was extracted with  $\text{Et}_2\text{O}$  (2x). The organic fractions were combined and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated by column chromatography ( $\text{SiO}_2$ , 15% EtOAc in hexanes, 1.0 g, 72% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.35-7.29 (m, 10H), 5.40 (dd, 1H,  $J_1 = 5.2$  Hz,  $J_2 = 2.8$  Hz), 5.24 (d, 1H,  $J = 12.4$  Hz), 5.18 (d, 1H,  $J = 12.0$  Hz), 4.61 (d, 1H,  $J = 12.0$  Hz), 4.53 (d, 1H,  $J = 12.0$  Hz), 4.41

(q, 1H,  $J = 7.2$  Hz), 3.96 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 5.2$  Hz), 3.84 (dd, 1H,  $J_1 = 10.8$  Hz,  $J_2 = 2.8$  Hz) 2.85 (s, 1H), 1.49 (d, 3H,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.27, 167.42, 137.45, 135.08, 128.77, 128.71, 128.62, 128.47, 128.42, 128.07, 127.83, 73.61, 72.95, 68.66, 67.65, 66.91, 20.65; HRMS ( $\text{M}+\text{Na}^+$ ) calc. 381.1314, found 381.1300.



**S(Bn)L.** (JLiii-36-1) Pd/C (31 mg, 10%) was added to a solution of **Bn-S(Bn)L** (1.0 g, 2.8 mmol) and  $\text{Et}_3\text{N}$  (0.2 ml, 1.4 mmol) in EtOAc (30 ml) under nitrogen. The flask was purged and stirred overnight under  $\text{H}_2$  (1 atm). The mixture was purged by nitrogen and filtered with

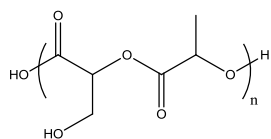
celite. The filtrate was washed with brine (acidified by HCl, 2x) and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated after concentration (0.57 g, 75% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38-7.28 (m, 5H), 5.38 (dd, 1H,  $J_1 = 5.2$  Hz,  $J_2 = 2.8$  Hz), 4.64 (d, 1H,  $J = 12.4$  Hz), 4.59 (d, 1H,  $J = 12.4$  Hz), 4.43 (q, 1H,  $J = 7.2$  Hz), 3.96 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 5.6$  Hz), 3.86 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 2.8$  Hz), 1.53 (d, 1H,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.22, 172.19, 137.23, 128.75, 128.27, 128.00, 73.80, 72.42, 68.39, 67.01, 20.66; HRMS ( $\text{M}+\text{Na}^+$ ) calc. 291.0845, found 291.0825.



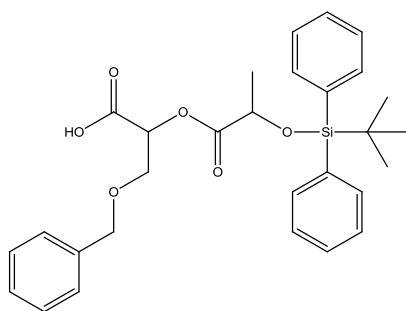
**Poly S(Bn)L.** (JLiii-37-2) DIC (0.5 ml, 3.2 mmol) was added dropwise to a solution of **S(Bn)L** (0.56 g, 2.1 mmol) and DPTS (0.13 g, 0.4 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.7 ml) in an ice bath. The mixture was then stirred for 3 h under RT. The product was precipitated in methanol

(75 ml) and stirred for 45 min. The precipitate was filtered and dried under vacuum to give a white powder (0.3 g, 57% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33-7.28 (m, 5H), 5.34 (dd, 1H,  $J_1 = 5.2$  Hz,  $J_2 = 3.2$  Hz), 5.27 (q, 1H,  $J = 7.2$  Hz), 4.59 (d, 1H,  $J = 12.4$  Hz), 4.55 (d, 1H,  $J = 12.0$  Hz), 3.96-3.89 (m, 2H), 1.60 (d, 3H,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  169.54,

166.89, 137.68, 128.61, 128.00, 127.92, 73.65, 72.78, 69.55, 68.63, 17.05; SEC (THF)  $M_n$  – 10.0 kDa; PDI – 1.74.

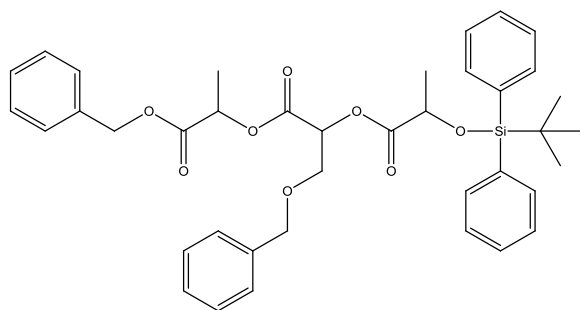


**Poly S(OH)L.** (JLiii-39-2) Pd/C (5 mg, 10%) was added to a solution of **Poly S(Bn)L** (10 mg) in DMF (0.5 ml) under nitrogen. The flask was purged and stirred overnight under  $H_2$  (1 atm). The mixture was purged by nitrogen and the solids were removed using a syringe filter (0.45  $\mu$ m). The filtrate was concentrated by vacuum overnight to give white powder (7 mg, 100% yield, 65% deprotected).  $^1H$  NMR (700 MHz, DMSO)  $\delta$  5.31-5.27 (m, 1H), 5.25 (q, 1H,  $J$  = 7.0 Hz), 5.15 (dd, 1H,  $J_1$  = 5.6 Hz,  $J_2$  = 2.1 Hz), 3.87 -3.84 (m, 1H), 3.78-3.74 (m, 1H), 1.50-1.47 (m, 3H) ;  $^{13}C$  NMR (100 MHz, DMSO)  $\delta$  169.3, 166.8, 166.5, 74.3, 68.8, 60.4, 16.6; SEC (DMF):  $M_n$  – 17.7 kDa, PDI – 1.1.



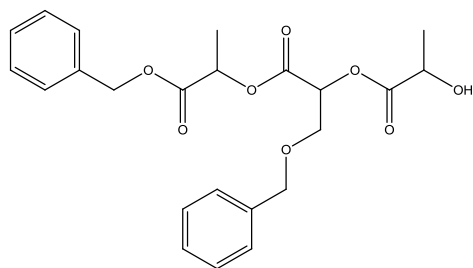
**S(Bn)L-Si.** (JLiii-36-2) Pd/C (74 mg, 10%) was added to a solution of **Bn-S(Bn)L-Si** (4.0 g, 6.7 mmol) and  $Et_3N$  (0.5 ml, 3.4 mmol) in EtOAc (75 ml) under nitrogen. The flask was purged and stirred overnight under  $H_2$  (1 atm). The mixture was purged with nitrogen and filtered through celite. The filtrate was washed with brine (acidified by HCl, 2x) and dried with  $MgSO_4$ . The mixture was filtered and concentrated. A clear oil was isolated by column chromatography ( $SiO_2$ , 15% EtOAc in hexanes, 1.9 g, 56% yield).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.70-7.67 (m, 5H), 7.44-7.25 (m, 10H), 5.14 (dd, 1H,  $J_1$  = 4.8 Hz,  $J_2$  = 2.8 Hz), 4.54 (d, 1H,  $J$  = 12.0 Hz), 4.48 (d, 1H,  $J$  = 12.4 Hz), 4.40 (q, 1H,  $J$  = 7.2 Hz), 3.82 (dd, 1H,  $J_1$  = 10.8 Hz,  $J_2$  = 5.2 Hz), 3.60 (dd, 1H,  $J_1$  = 10.8 Hz,  $J_2$  = 2.8 Hz), 1.44 (d, 3H,  $J$  = 7.2 Hz), 1.10 (s, 9H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  173.25, 172.80, 137.43, 136.17, 135.98, 133.59, 133.20, 130.03, 130.01, 128.63, 128.07, 127.92, 127.88,

127.80, 73.67, 71.77, 68.76, 68.51, 26.99, 21.43, 19.42; HRMS ( $M+Na^+$ ) calc. 529.2022, found 529.2031.



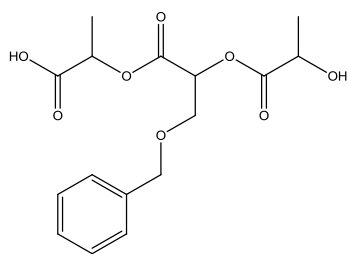
**Bn-LS(Bn)L-Si.** (JLiii-37-1) DCC (0.9 g, 3.7 mmol) was added to a solution of **Bn-L** (0.7 g, 3.7 mmol), **S(Bn)L-Si** (1.9 g, 3.7 mmol) and DMAP (0.3 g, 0.9 mmol) in  $CH_2Cl_2$  (40 ml). The mixture was stirred for 4 h and filtered to remove

the DCU. The filtrate was concentrated and a clear oil was isolated by flash chromatography ( $SiO_2$ , 5% EtOAc in hexanes, 1.9 g, 74% yield).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.71-7.27 (m, 20H), 5.23 (q, 1H,  $J = 7.2$  Hz), 5.20 (dd, 1H,  $J_1 = 6.0$  Hz,  $J_2 = 3.2$  Hz), 5.17 (d, 1H,  $J = 12.4$  Hz), 5.13 (d, 1H,  $J = 12.0$  Hz), 4.48 (d, 1H,  $J = 12.0$  Hz), 4.45 (d, 1H,  $J = 12.0$  Hz), 4.41 (q, 1H,  $J = 6.8$  Hz), 3.77 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 6.0$  Hz), 3.73 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 3.2$  Hz), 1.52 (d, 3H,  $J = 7.2$  Hz), 1.45 (d, 3H,  $J = 6.8$  Hz), 1.11 (s, 9H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  173.35, 170.02, 167.33, 137.79, 136.13, 136.05, 135.96, 135.34, 133.67, 133.21, 129.99, 129.98, 128.88, 128.81, 128.77, 128.67, 128.62, 128.52, 128.45, 128.01, 127.89, 127.86, 127.81, 73.52, 72.19, 69.66, 68.77, 68.70, 67.34, 26.99, 21.50, 19.42, 17.06; HRMS ( $M+Na^+$ ) calc. 691.2703, found 691.2722.



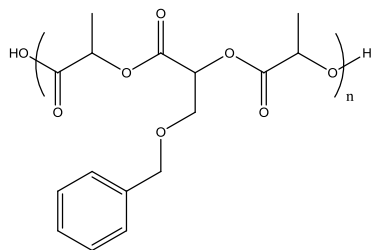
**Bn-LS(Bn)L.** (JLiii-38-1) TBAF (4 ml, 4 mmol, 1M in THF) was added to a solution of **Bn-LS(Bn)L-Si** (1.8 g, 2.7 mmol) and acetic acid (0.3 ml, 4.8 mmol) in THF (30 ml). The mixture was stirred for 2 h and washed with  $NaHCO_3$  (2x) and brine (1x). The aqueous solution was extracted with  $Et_2O$  (2x). The

organic fractions were combined and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated by column chromatography ( $\text{SiO}_2$ , 15% EtOAc in hexanes, 0.8 g, 68% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38-7.30 (m, 10H), 5.40 (dd, 1H,  $J_1 = 6.8$  Hz,  $J_2 = 3.2$  Hz), 5.24 (q, 1H,  $J = 7.2$  Hz), 5.18 (d, 1H,  $J = 12.0$  Hz), 5.14 (d, 1H,  $J = 12.0$  Hz), 4.57 (d, 1H,  $J = 11.6$  Hz), 4.54 (d, 1H,  $J = 12.4$  Hz), 4.41 (p, 1H,  $J = 7.2$  Hz), 3.93 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 2.8$  Hz), 3.87 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 6.4$  Hz), 2.70 (d, 1H,  $J = 6.0$  Hz), 1.54 (d, 3H,  $J = 7.2$  Hz), 1.52 (d, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.34, 169.90, 167.02, 137.59, 135.25, 128.85, 128.76, 128.65, 128.50, 128.46, 128.18, 128.09, 127.87, 73.66, 72.88, 69.88, 68.71, 67.48, 66.93, 20.72, 17.05; HRMS ( $\text{M}+\text{Na}^+$ ) calc. 435.1525, found 453.1493.



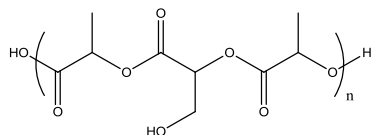
**LS(Bn)L.** (JLiii-38-2) Pd/C (20 mg, 10%) was added to a solution of **Bn-LS(Bn)L** (0.65 g, 1.5 mmol) and  $\text{Et}_3\text{N}$  (0.1 ml, 0.95 mmol) in EtOAc (20 ml) under nitrogen. The flask was purged and stirred overnight under  $\text{H}_2$  (1 atm). The mixture was purged by nitrogen and filtered with celite. The filtrate was washed with brine (acidified by HCl, 2x) and dried with  $\text{MgSO}_4$ . The mixture was filtered and concentrated. A clear oil was isolated after concentration (0.25 g, 50% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.36-7.29 (m, 5H), 5.40 (dd, 1H,  $J_1 = 5.6$  Hz,  $J_2 = 2.8$  Hz), 5.25 (q, 1H,  $J = 7.2$  Hz), 4.63 (d, 1H,  $J = 12.4$  Hz), 4.59 (d, 1H,  $J = 12.0$  Hz), 4.42 (q, 1H,  $J = 6.8$  Hz), 3.98 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 6.0$  Hz), 3.92 (dd, 1H,  $J_1 = 11.2$  Hz,  $J_2 = 3.2$  Hz), 1.57 (d, 3H,  $J = 6.8$  Hz), 1.53 (d, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.35, 167.08, 137.30, 128.72, 128.24, 128.01, 73.84, 72.78, 68.64, 67.00, 20.68, 17.02; HRMS ( $\text{M}+\text{Na}^+$ ) calc. 363.1056, found 363.1039.





**Poly LS(Bn)L**. (JLiii-39-1) DIC (0.16 ml, 0.6 mmol) was added dropwise to a solution of **LS(Bn)L** (0.23 g, 0.7 mmol) and DPTS (0.04 g, 0.00014 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.22 ml) in an ice bath.

The mixture was then stirred for 3 h under RT. The product was precipitated in methanol (15 ml) and stirred for 45 min. The precipitate was filtered and dried under vacuum to give a white powder (0.14 g, 64% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.33-7.28 (m, 5H), 5.34 (dd, 1H, *J*<sub>1</sub> = 5.6 Hz, *J*<sub>2</sub> = 2.8 Hz), 5.23 (q, 1H, *J* = 7.2 Hz), 5.20 (q, 1H, *J* = 7.2 Hz), 4.60 (d, 1H, *J* = 12.4 Hz), 4.56 (d, 1H, *J* = 12.4 Hz), 3.95 (dd, 1H, *J*<sub>1</sub> = 11.2 Hz, *J*<sub>2</sub> = 2.8 Hz), 3.91 (dd, 1H, *J*<sub>1</sub> = 11.2 Hz, *J*<sub>2</sub> = 6.0 Hz), 1.59 (d, 3H, *J* = 7.6 Hz), 1.57 (d, 3H, *J* = 7.2 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.81, 169.44, 167.01, 137.66, 128.62, 128.01, 127.93, 73.68, 72.80, 69.60, 69.16, 68.66, 16.94; SEC (THF): M<sub>n</sub> – 21.3 kDa, PDI - 1.73.



**Poly LS(OH)L** (JLiii-44-1) Pd/C (5 mg, 10%) was added to a solution of **Poly LS(Bn)L** (10 mg) in DMF (0.5 ml) under nitrogen. The flask was purged and stirred overnight under H<sub>2</sub> (1 atm). The mixture was purged by nitrogen and filtered with syringe filter (0.45 μm). The filtrate was concentrated by vacuum overnight to give white powder (8 mg, 100% yield, 85% deprotected). <sup>1</sup>H NMR (700 MHz, DMSO), d 5.42(t, 1H, *J* = 5.6 Hz), 5.25-5.20 (m, 2H), 5.15 (dd, 1H, *J*<sub>1</sub> = 6.3 Hz, *J*<sub>2</sub> = 2.8 Hz), 3.84 (m, 1H), 3.76 (m, 1H), 1.49 (d, 3H, *J* = 7.0 Hz), 1.46 (d, 3H, *J* = 7.0 Hz); <sup>13</sup>C NMR (100 MHz, DMSO), d 169.4, 169.0, 166.9, 74.2, 68.8, 68.6, 60.4, 16.5, 16.4; SEC (DMF): M<sub>n</sub> – 47.5 kDa, PDI – 1.2.

## 2.4 NAMING CONVENTION

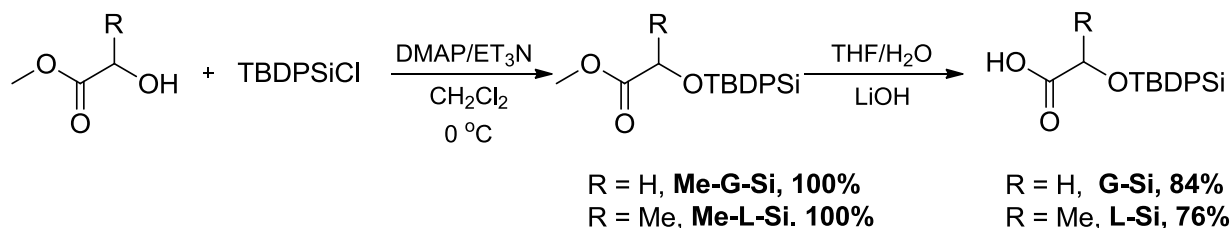
The abbreviation of monomers and protection groups used here are listed in Table 1. Segmers are named by listing the monomers from carboxylic acid side (*C*-side) to the hydroxyl side (*O*-side) in sequence order. Polymers are named by adding **Poly** as a prefix. For instance, GLG is the segmer with glycolic acid, L-lactic acid and glycolic acid from *C*-side to *O*-side. The polymer prepared from the GLG segmer is named **Poly GLG**. Using this convention, the polymer prepared from segmer LGG would be named **Poly LGG** despite the fact that **Poly GLG** and **Poly LGG** differ only in which monomers are located at the chain ends.

**Table 1.** Naming conventions for segmers and polymers

Symbol	Definition
<b>L</b>	L-Lactic acid unit ( <i>S</i> configuration)
<b>L<sub>rac</sub></b>	D,L-Lactic acid unit (racemic configuration)
<b>G</b>	Glycolic acid unit
<b>Bn</b>	Terminal benzyl protecting group
<b>TBDPSi</b>	Terminal silyl protecting group (tert-butyldiphenylsilyl group)
<b>Si</b>	Terminal silyl protecting group (tert-butyldiphenylsilyl group)
<b>R</b>	Random sequenced
<b>ROP</b>	Polymer prepared by ring opening polymerization
<b>SAP</b>	Polymer prepared by segmer assembly polymerization
<b>S(Bn)</b>	S-3-Benzoyloxy-2-hydroxypropionic acid unit
<b>S(OH)</b>	S-2,3-Dihydroxypropionic acid unit

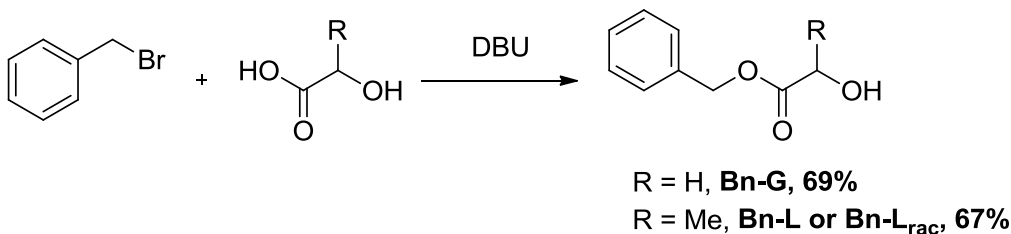
## 2.5 RESULTS AND DISCUSSION

### 2.5.1 PLGA Synthesis



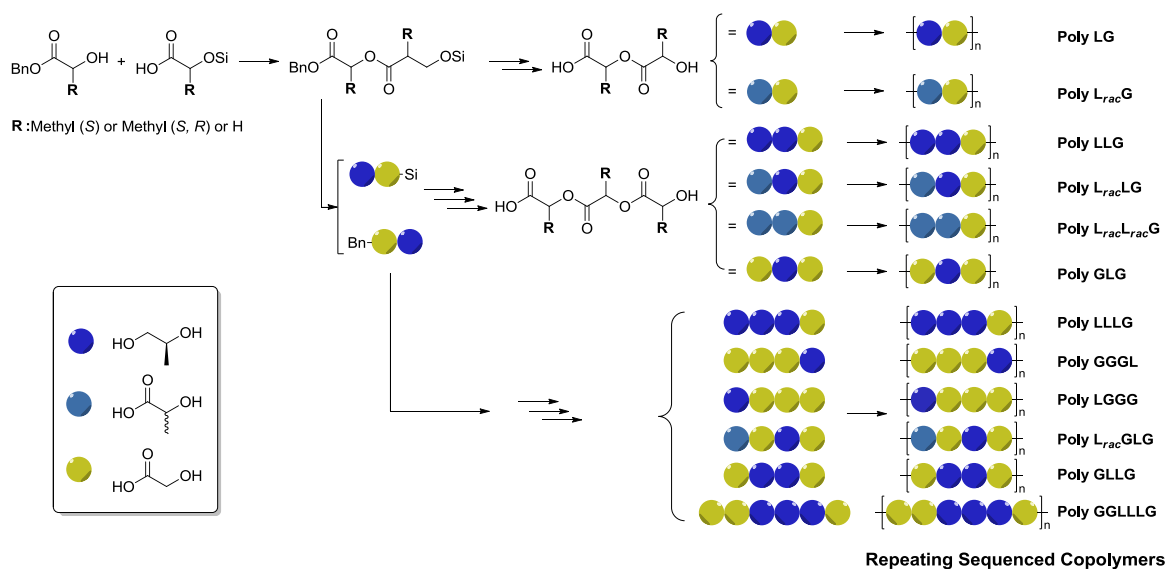
**Figure 7.** Preparation of building block molecules with TBDPSi protecting groups

Five key building blocks were prepared by the protection of lactic and glycolic acids. The alcohol functional group of both glycolic and lactic acids was protected with *tert*-butyldiphenylsilyl (TBDPSi). Reaction of commercially available methyl glycolate with TBDPSiCl in the presence of DMAP and Et<sub>3</sub>N gave the silyl protected methyl ester (Me-G-Si) in 100% yield. Saponification of the ester with LiOH gave silyl-protected glycolic acid (G-Si) in 84% yield. The choice of TBDPSi over other similar silane protection groups such as *tert*-butyldimethylsilane (TBDMSi) was based on the need to stabilize the protecting group against the basic conditions required for the saponification step. The silyl protected lactic acid (L-Si) was produced analogously but needed a longer saponification (Figure 7). Both products were verified by comparison of the <sup>1</sup>H NMR spectra with literature reports.<sup>81</sup>



**Figure 8.** Preparation of building block molecules with benzyl protecting groups

The acid functional group of both glycolic, L-lactic and *rac*-lactic acids was protected with the chemically orthogonal benzyl group in anticipation of future protection group manipulations. Reaction of glycolic acid with benzyl bromide in the presence of DBU in benzene gave benzyl protected glycolic acid (Bn-G) in 69% yield after chromatography. The protection of the lactic acid required slightly different conditions because the purchased acid was a 90% solution in water. The water was first removed by distillation after addition of DBU. Reaction with benzyl bromide then took place in DMF solvent to give, after vacuum distillation, the product (Bn-L) as clear oil in 67% yield (Figure 8). Both compounds were verified by comparison of the  $^1\text{H}$  NMR spectra with the literature report.<sup>81</sup>



**Figure 9.** Segmer assembly polymerization synthesis of PLGA copolymers

The first RSC of PLGA, **Poly LG**, was prepared by using Bn-L and G-Si as the building blocks (Figure 9). Coupling of the two units in the presence of DCC and DMAP in  $\text{CH}_2\text{Cl}_2$  gave orthogonally protected Bn-LG-Si in 68% yield after purification by column chromatography. The two protecting groups were then removed. Reaction of Bn-LG-Si with TBAF in the presence of acetic acid in THF cleaved the TBDPSi selectively and gave Bn-LG as product in 75% yield.

The benzyl ester was subsequently deprotected by hydrogenation in the presence of Pd/C as catalyst. The solvent was removed *in vacuo* overnight then the segment LG was diluted with dry CH<sub>2</sub>Cl<sub>2</sub> and polymerized in the presence of DIC as condensation reagent and DPTS as catalyst. The polymer was purified by precipitation in methanol to remove the catalyst and the side product. The product was characterized by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy for structure confirmation and SEC for molecular weight determination. By using the Bn-L<sub>rac</sub> to substitute Bn-L, the alternating copolymer **Poly L<sub>rac</sub>G** with racemic stereochemical centers was prepared.

Selective deprotection of the di-protected units yielded building blocks for longer segments. The three benzyl protected building blocks, Bn-LG, Bn-GL and Bn-GG, were thus prepared by removal of the TBDPSi group in TBAF/AcOH/THF solution from the deprotected precursor. Bn-LG and Bn-GL were purified by column chromatography while Bn-GG was purified by precipitating the oil-like mixture in hexanes because Bn-GG is highly crystalline. Bn-LL was prepared by a one-step ring opening of L,L-lactide with benzyl alcohol and DMAP catalyst. The Bn-LL was then purified by vacuum distillation. Silyl protected building blocks were prepared by removing the benzyl group by hydrogenolysis of the di-protected dimers with Pd/C. LG-Si, GL-Si, LL-Si, GL-Si and L<sub>rac</sub>G-Si were thus prepared. The general yields of the preparations were 70-90%. All building blocks were characterized by NMR spectroscopy and mass spectrometry to confirm the chemical structure.

Trimeric, tetrameric and hexameric segments were prepared by coupling-deprotecting steps using those longer building blocks. To prepare trimeric segment LLG, for example, Bn-LLG-Si was first prepared by coupling Bn-L and LG-Si using the DCC/DMAP method to give a 77% yield of Bn-LLG-Si. The TBDPSi group was cleaved in TBAF/AcOH/THF solution and the benzyl group was subsequently deprotected by hydrogenolysis over Pd/C. The overall yield over

the two steps was 72%. The same procedure was used to prepare other trimeric segmers including  $L_{rac}L_{rac}G$ ,  $L_{rac}LG$  and  $GLG$ .

The tetramer oligomer  $L_{rac}GLG$  was prepared in 80% yield by the coupling of  $Bn-L_{rac}G$  and  $LG-Si$ . The orthogonally protected oligomer was then treated with TBAF to cleave TBDPSi and subsequently hydrogenated in the presence of Pd/C to give the finally oligomer  $L_{rac}GLG$  with both carboxylic acid group and alcohol group deprotected in 82% yield. The de-protected oligomer was then polymerized as described previously and isolated by precipitation in methanol to give pure poly  $L_{rac}GLG$  (Figure 9). Using this procedure, other oligomers including  $LLLG$ ,  $GLLL$ ,  $GLLG$  and  $LGGG$  were prepared.  $LGGG$ , which has three glycolic units in the segmer, required particularly gentle conditions because the ester bonds of glycolic units are easy to cleave. Both the coupling of building blocks  $Bn-LG$  and  $GG-Si$  and the cleavage of TBDPSi group were carried out for shorter reaction times than for other oligomers. Even with the addition of more acetic acid as buffer and shorter reaction times,  $Bn-LG$  was still isolated from reaction mixture which is consistent with decomposition as proof of cleavage.

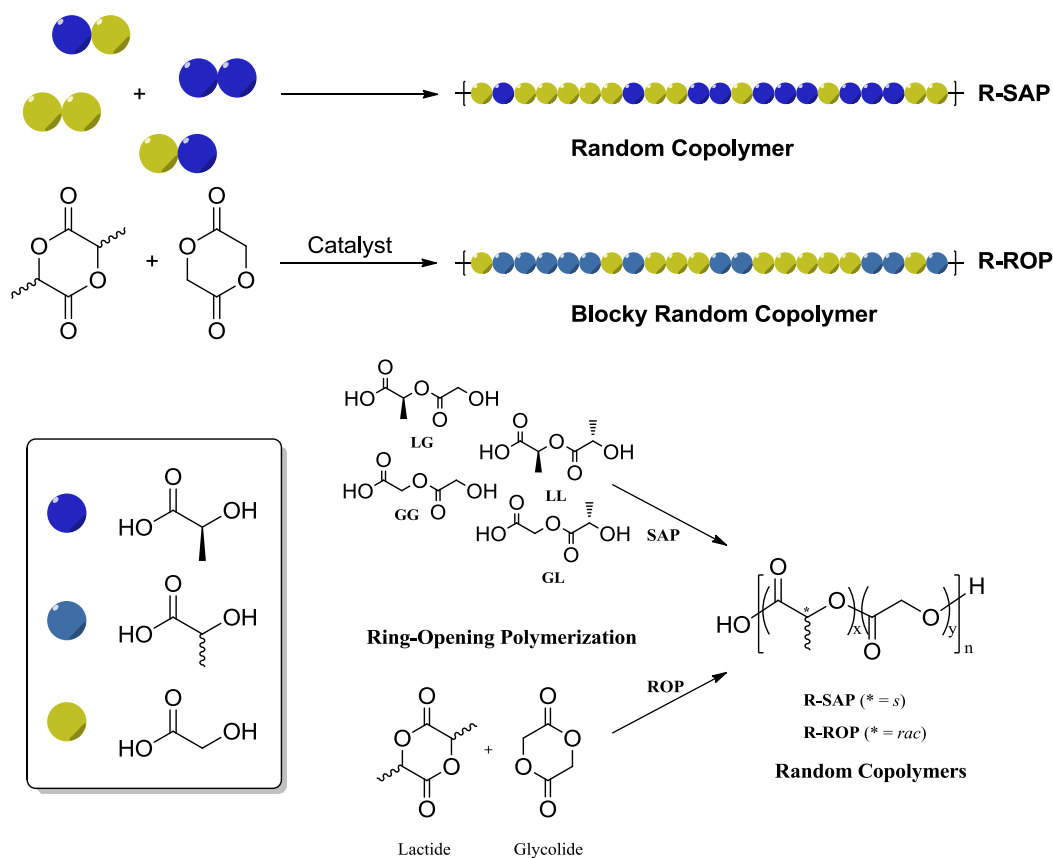
The hexameric segmer,  $GGLLLG$ , was also prepared using the coupling-deprotecting approach.  $Bn-GGLLLG-Si$  was first prepared by the coupling of  $Bn-GG$  and  $LLLG-Si$ . The TBDPSi group was carefully deprotected by TBAF with large quantity of AcOH (8x) and shorter reaction time to avoid decomposition of the product. The benzyl group was then removed by hydrogenolysis with Pd/C as the catalyst. The longer segmer proved more sensitive to the deprotection conditions and required an extra purification step to remove decomposition products.

The polymers of above all oligomers were prepared and were characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy for structure confirmation and SEC for molecular weight determination. A list of PLGAs prepared is shown in Table 2.

**Table 2.** Characterization data for sequenced PLGAs

	$M_n^a$ (kDa)	$M_w$ (kDa)	PDI
<b>Poly LG</b>	37.2	52.1	1.4
<b>Poly <math>L_{rac}</math>G</b>	30.0	38.4	1.3
<b>Poly LLG</b>	45.7	68.6	1.5
<b>Poly <math>L_{rac}</math>LG</b>	31.0	45.3	1.5
<b>Poly <math>L_{rac}L_{rac}</math>G</b>	30.0	38.7	1.3
<b>Poly GLG</b>	24.3	35.2	1.4
<b>Poly <math>L_{rac}</math>GLG</b>	12.4	17.7	1.4
<b>Poly LLLG</b>	10.4	18.7	1.8
<b>Poly GLLL</b>	8.7	13.5	1.6
<b>Poly LGGG</b>	7.8	9.8	1.3
<b>Poly GLLG</b>	35.0	41.3	1.2
<b>Poly GGLLLG</b>	45.9	52.8	1.1

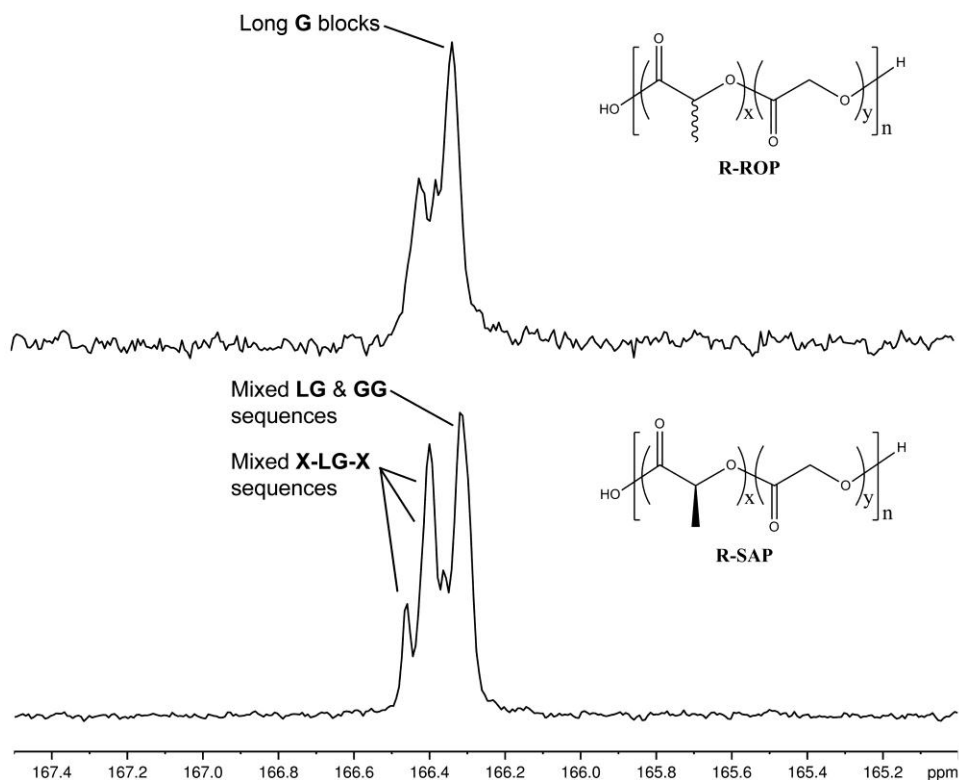
<sup>a</sup>determined by SEC (THF) relative to polystyrene standards.



**Figure 10.** Synthetic approach to random PLGA copolymers using both segment assembly polymerization (SAP) and ring-opening polymerization (ROP).

The SAP method can be used not only to prepare the RSCs of PLGA, but also to prepare random PLGA by mixing selected segmers. A random 1:1 L:G PLGA sample was thus produced using the condensation reaction of the dimeric precursors LG, GG, LL, and GL in equal proportions. It should be noted that this second polymer, which is termed **R-SAP**, was synthesized from stereopure L-lactic precursors and prepared with the same reaction conditions as described above.  $^1\text{H}$  NMR spectroscopy of the polymers confirmed that all samples consist of a 1:1 ratio of L to G.





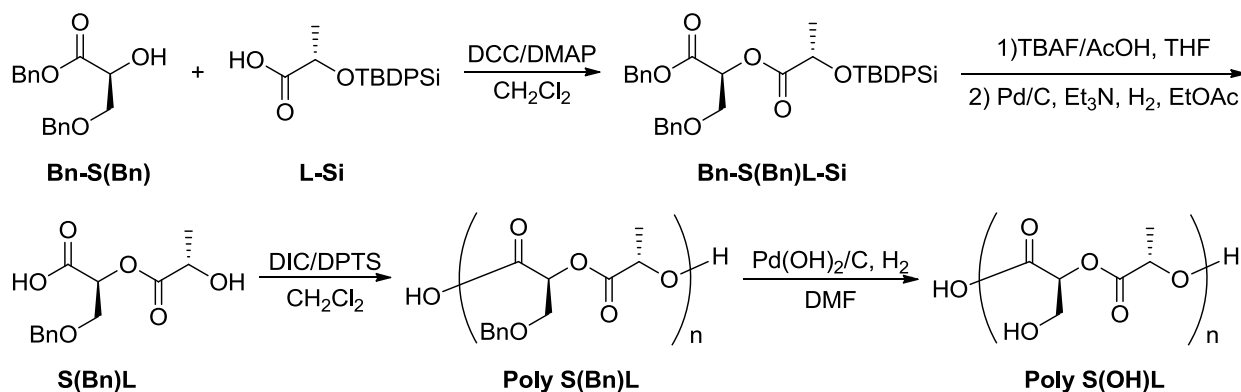
**Figure 11.** Comparison of glycolyl carbonyl resonances for R-ROP (top) and R-SAP (bottom). X represents either L or G units.

A comparison of the glycolyl carbonyl region of a random PLGA with 1:1 ratio of L:G (**R-ROP 50**, prepared by Durect Corp. using ROP) and **R-SAP 50** random copolymers highlights differences in their microstructures. Although both polymers exhibit several overlapping resonances near  $\delta$  166.4,<sup>85</sup> the relative ratios of the individual peaks differ significantly. For example, the resonance at  $\delta$  166.33 in **R-ROP 50** is more intense than that at  $\delta$  166.42. The spectrum of **R-SAP 50** shows both peaks are similar in intensity (Figure 11). Previous studies<sup>14,49</sup> have suggested that the  $\delta$  166.33 resonance is associated with the glycolyl carbonyls of units located in the center of pure G blocks while that at  $\delta$  166.42 is characteristic of a glycolyl carbonyls with nearby L units. The relative ratios of these peaks are consistent the microstructural trends that are expected from the two distinct synthetic approaches. The **R-ROP**

**50** polymer, prepared by ring-opening of a 50:50 mixture of glycolide and lactide, are known to possess blocks of pure G.<sup>29</sup> In contrast the **R-SAP 50** copolymer, produced by the condensation of four dimers, should give statistically fewer runs of pure G unless the reaction rate of GG with other GG units is significantly higher than that with other units. Based on our extensive experience in handling these dimers, differences in reaction rates between dimers are not large.

### 2.5.2 Side chain modification

Side chain modification of PLGA/PLA is very important for the application of PLGAs in the biomaterials field. Installation of reactive side groups allow for the post-polymerization attachment of biomedically relevant substituents. Post-polymerization modification of biodegradable and bioassimilable polymers has proved useful in both tissue engineering and drug delivery applications.<sup>86-91</sup> The introduction of such side chains in ROP-prepared PLA or PLGA polymers is generally accomplished through the addition of derivatized lactide monomers to the polymerization mixture. This method gives a random distribution of the functional groups along the copolymer chain.



**Figure 12.** Synthesis approach of side chain modified RSC of PLGA

By using a SAP approach, however, it is possible to create RSCs wherein the functional groups are evenly distributed throughout the polymer chain. The general approach to these RSCs convergent, and is similar to the preparation of PLGA RSCs described above. The project was a collaborative effort between Dr. Ryan Stayshich, Ryan Weiss and the author of this dissertation and the results were published.<sup>76</sup> There are six polymers prepared in total for this project and two of them are prepared by the author of this dissertation, e.g. Figure 12. The hydroxyl modified unit is abbreviated S, because it is derived from the amino acid, serine. The S(Bn) monomer, which was prepared following the procedure by Kelly and Weck groups,<sup>92-94</sup> was coupled with L-Si to give the di-protected dimer in a 78% yield. Deprotection of benzyl group on the carboxylic acid side by catalytic hydrogenolysis produced the S(Bn)L-Si in a 56% yield. The trimer Bn-LS(Bn)L-Si was prepared by coupling of Bn-L and S(Bn)L-Si in a 74% yield. The protecting groups were removed from the dimer and trimer to give segmers S(Bn)L and LS(Bn)L in yields of 54% and 34%, respectively.

**Table 3.** Characterization data PLGAs bearing protected and deprotected hydroxyl side chains

	M <sub>n</sub> (kDa)	M <sub>w</sub> (kDa)	PDI
<b>Poly S(Bn)L</b>	10.0 <sup>a</sup>	17.4	1.7
<b>Poly LS(OH)L</b>	17.7 <sup>b</sup>	19.5	1.1
<b>Poly LS(Bn)L</b>	21.3 <sup>a</sup>	36.8	1.7
<b>Poly LS(OH)L</b>	47.5 <sup>b</sup>	57.0	1.2

<sup>a</sup>Determined by SEC (THF) relative to polystyrene standards; <sup>b</sup>Determined by SEC (DMF) relative to polystyrene standards.

The RSCs of these two segmers were prepared using our standard DIC/DTPS method. The polymers exhibited moderate molecular weights: **Poly S(Bn)L** which had a M<sub>n</sub> of 10.0 kDa by SEC in THF and 36.8 kDa by SEC in DMF and **Poly LS(Bn)L** which had a M<sub>n</sub> of 21.3 kDa

by SEC in THF and 50.8 kDa by SEC in DMF. Post-polymerization removal of the benzyl protecting group from the side chain was performed in DMF because DMF was a good solvent for the polymer both before and after hydrogenolysis which is important because it minimizes the activation enthalpy between the benzyl-protected polymers and the more polar transition state.<sup>76</sup> De-protection of benzyl groups gave **Poly S(OH)L** and **Poly LS(OH)L** in 65% and 85% yields, respectively. The  $M_n$ s of the two polymers were 17.7 kDa and 47.5 kDa by SEC (DMF) (see Table 3).

### 2.5.3 Optimization of polymerization conditions

#### 2.5.3.1 Effect of coupling reagent choice on polymer molecular weight

During the course of this project significant effort was dedicated to improving the polymerization to give high molecular weight polymers. Condensation polymerizations are challenging because they do not achieve polymer level molecular weights until they have reached very high degrees of conversion (> 99%) and because there are no easy ways to select a desired molecular weight. It is, therefore, necessary to optimize all aspects of the polymerization. Although we implemented many strategies such as maximizing concentration, one of the most important modifications that we made during the course of our development, was changing coupling conditions. Initially, we employed the widely used esterification agent *N,N'*-dicyclohexylcarbodiimide (DCC) and the catalyst 4-dimethylamine-pyridine (DMAP).<sup>81</sup> With time, however, both Dr. Ryan Stayshich and the author of this dissertation established that these conditions caused segment and polymer degradation. We hypothesize that the basicity of the DMAP is a significant factor, as the nitrogen may be attacking the esters and promoting cleavage and/or transesterification. We also found that the addition of DCC solid into the polymerization

container is technically challenging as the DCC a solid and is quite sensitive to atmospheric moisture. Molecular weights of polymers produced by this method were often low and the reactions were not reproducible.

To optimize the reaction DCC was also substituted with a liquid coupling reagent—*N,N'*-diisopropylcarbodiimide (DIC).<sup>37</sup> The addition of DIC can be completed by using syringe by which a slow and steady addition can be achieved. To reduce the basicity of the DMAP, we used the strategy developed by Moore which involves the addition of toluene sulfuric acid to neutralize the DMAP. The neutralized product is 4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS).<sup>37</sup> The DPTS is milder with regard to the ester bonds but is still an effective catalyst. With these changes in polymerization conditions, we were able to routinely achieve molecular weights greater than 30 kDa.

### 2.5.3.2 Effect of incomplete deprotection

Despite our efforts to optimize the polymerization, the  $M_n$ s of our PLGA RSCs in some cases are still low, around 10 kDa. Although strict control of concentration did help improve the yield in some cases, some reactions still failed to give polymer in our target molecular weight range of > 20 kDa.

To understand the source of the difficulty, MALDI-ToF-MS was employed to analyze the chain structure of the “good” RSC and “bad” RSCs. Two **Poly LGGs** were chosen for study. As the molecular weights from SEC (THF) are 24.2 kDa and 11.1 kDa the two **Poly LGGs** are named as **Poly LGG24** and **Poly LGG11**, respectively.

#### Equation 1

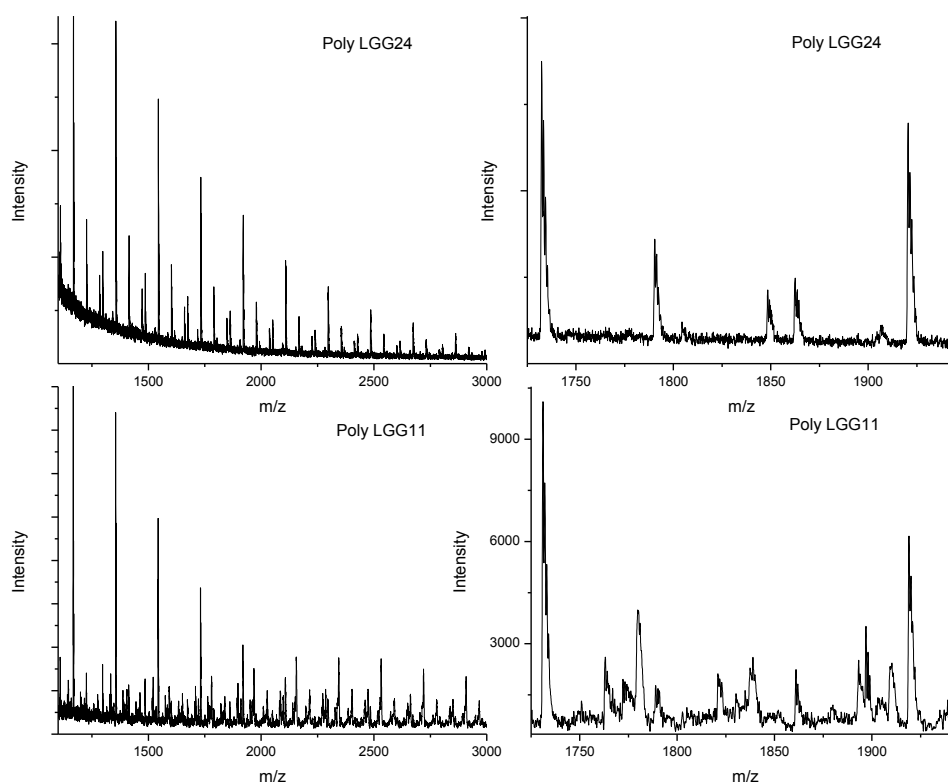
$$m = xM_{GLG} + yM_G + zM_L + E_I + E_{II} + \cdots + M^+$$

The  $m/z$  peaks from the spectra were assigned in Equation 1 by using the equation.  $xM_{GLG}$  is the number and the mass of the repeating LGG unit,  $yM_G$  and  $zM_L$  are the number and the mass of glycolic unit and lactic unit other than the repeating units. If there is any error on the polymer chain, one or two extra L or G groups can be found, so that  $y$  and  $z$  can be 0, 1 or 2 only.  $E_I$  and  $E_{II}$  are the molar masses of possible end groups on each side of the polymer. As potassium is the cation that is added to the MALDI-ToF samples, the  $M^+$  in most cases is considered as  $K^+$  and the molar mass is 39.

The MALDI-ToF spectra of the two **Poly LGGs** are shown in Figure 13. It is important to note that the distribution of molecular weights present in the spectrum is not representative of the molecular weight envelop for the sample as the ionization/volatilization process favors shorter chains. For our analysis, we therefore focus on an  $m/z$  range (1100 Da to 3000 Da) which shows peaks of significant intensity for both samples. The peaks with the highest intensities in the spectrum are separated by 188 mass units, which is equal to the molar mass of the repeating unit—LGG. After analysis, these high intensity peaks are assigned as **Poly LGG** with cyclic structure plus one  $K^+$  (Unless explicitly mentioned  $K^+$  can be assumed as the counterion for all species discussed). In this condition,  $E_I$  and  $E_{II}$  are zero because there is no end groups for cyclic copolymer. The highest intensity peaks in the spectra are thus assigned as  $(LGG)_6$  to  $(LGG)_{15}$ . The presence of moderately large cyclic oligomers is well known for polyesters of the type we prepare.<sup>95</sup> Such cyclic species can be produced from either end group reactions of growing oligomers or “back-biting” transesterification.<sup>96</sup>

To reveal the difference between the two **Poly LGGs**, an expansion of the region between  $m/z$  1725 and 1945 is shown in Figure 13(right). This region comprises all species with molecular weights in the range of  $(LGG)_9$  and  $(LGG)_{10}$ . In Poly LGG24, we can see that there

are three peaks between the peaks of (LGG)<sub>9</sub> and (LGG)<sub>10</sub>. Those three peaks, after assignment, are confirmed as (LGG)<sub>10</sub> – G (= cyclic decamer missing one G unit), (LGG)<sub>10</sub> – L and (LGG)<sub>10</sub> – LG from  $m/z$  high to low, respectively. The structure of these polymers is still cyclic. The presence of the “error” peaks for incomplete sequences establishes that there is some transesterification during the reaction. In the case of the cyclics, a single error could easily be introduced during the ring-closing if it arose from a “back-biting” event from a longer chain. Transesterification could, of course, also occur between linear chains. Although the transesterification errors are visible, the repeating LGG sequence predominates, however.



**Figure 13.** MALDI-ToF-MS spectra of Poly LGG24 and Poly LGG11

In the spectrum of the lower molecular weight, **Poly LGG11**, there are peaks for species that are not present in the spectrum for **Poly LGG24**. The peaks with  $m/z$  is 1763, 1821 and 1893 are non-cyclic oligomers with carboxylic acid and hydroxyl end groups while those with

$m/z$  is 1779, 1839, 1897 and 1911 correspond to non-cyclic oligomers with benzyl and hydroxyl end groups. The exact assignments are summarized in Table 4.

**Table 4.** Assignment of MALDI-ToF-MS spectra ( $m/z$  range: 1725-1950).

Poly LGG24		Poly LGG11	
Peak ( $m/z$ )	Structure <sup>b</sup>	Peak ( $m/z$ )	Structure <sup>b</sup>
1731	Cy9 + K <sup>+</sup>	1731	Cy9 + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1763	Ch(HO-H)11 – G + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1779	Ch(Bn-H)10 – GG – GG + Na <sup>+</sup>
1789	Cy10 – LG + K <sup>+</sup>	1789	Cy10 – LG + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1821	Ch(HO-H)11 – GG + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1839	Ch(Bn-H)9 + K <sup>+</sup>
1847	Cy10 – L + K <sup>+</sup>	1847	Cy10 – L + K <sup>+</sup>
1861	Cy10 – G + K <sup>+</sup>	1861	Cy10 – G + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1893	Ch(HO-H)11 – GG – GG + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1897	Ch(Bn-H)10 – LG + K <sup>+</sup>
<sup>a</sup>	<sup>a</sup>	1911	Ch(Bn-H)10 – GG + K <sup>+</sup>
1919	Cy10 + K <sup>+</sup>	1919	Cy10 + K <sup>+</sup>

<sup>a</sup>no peak detected. <sup>b</sup>structure abbreviations are listed here, Cy is cyclic structure; Ch is chain structure; HO-H represents acid and alcohol terminated chain; Bn-H represents benzyl and alcohol terminated chain.

The MALDI-ToF spectra suggest that incomplete deprotection is responsible for the difference in molecular weights. For **Poly LGG11**, not only do we see peaks corresponding to benzyl-terminated oligomers but also peaks for other non-cyclic oligomers which is consistent



with the lower molecular weight distribution expected for a condensation polymerization carried out with a stoichiometric imbalance in reactive end-groups. In contrast, **Poly LGG24** exhibits peaks only for cyclic oligomers. This pattern is expected for the higher molecular weight sample, since the cyclic oligomers cannot grow once formed while the shorter linear oligomers will have been incorporated into longer chains.

The MALDI-ToF data provide direct evidence that we do not always achieve complete deprotection of our segmers under the hydrogenolysis conditions employed and suggest that the poor deprotection correlates with lower molecular weights. Although such incomplete deprotection appears to be relatively rare, in some cases such as **Poly GLLG** and **Poly GGLLLG**, the incomplete deprotection resulted in very low polymer molecular weights (~ 8-10 kDa).

To solve the problem of incomplete deprotection, the amount of Pd/C was increased from 10 to 15% equivalent w/w. For example, Bn-GLLG, which had under the original conditions showed incomplete deprotection and commensurate low molecular weight upon polymerization, changing the quantity of Pd catalyst gave a segmer whose post-polymerization  $M_n$  was 35 kDa. In some cases, however, these conditions still did not provide complete deprotection of the benzyl group. For **Poly GGLLLG**, however, we were able to remove the benzyl-protected segmer by column chromatography to eventually give a segmer that yield a polymer with an  $M_n$  of 46 kDa. It should be noted that column chromatography of the completely deprotected segmer is not generally a desirable approach as there is always some segmer degradation on the acidic silica.

## 2.6 CONCLUSIONS

A series of PLGA RSCs, including polymers bearing functional side-chains, were prepared by using Segmer Assembly Polymerization method. The molecular weight ranges from 8 kDa to 46 kDa. Two coupling methodologies DCC/DMAP and DIC/DPTS were used in the polymerization and the DIC/DPTS was shown to give higher molecular weight polymers. Incomplete deprotection of the benzyl protecting groups was also identified by MALDI-ToF as a factor in lowering polymer molecular weights.

### 3.0 THE DEPENDENCE OF MATERIAL PROPERTIES ON MONOMER SEQUENCE IN PLGA COPOLYMERS

#### 3.1 OVERVIEW

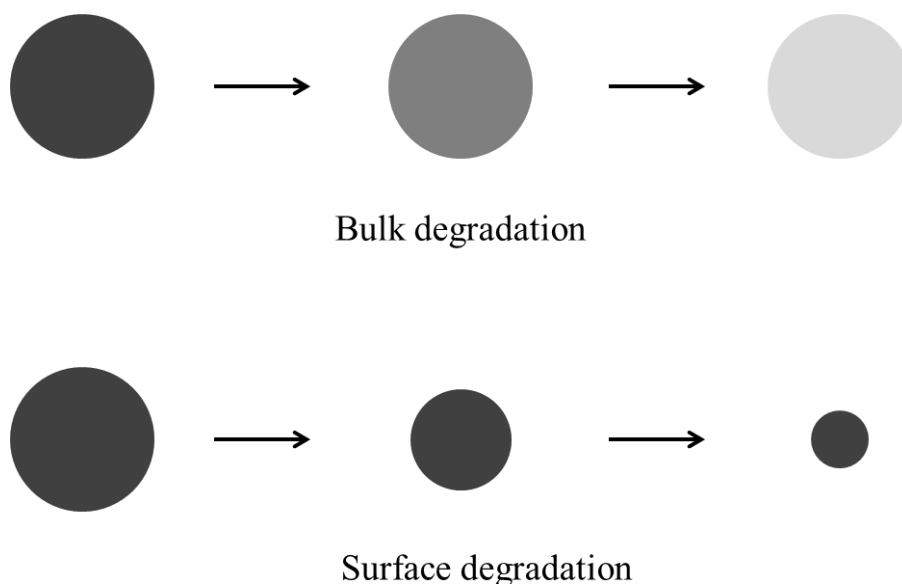
In this chapter the hydrolysis, thermal and *in vitro* drug delivery properties of a series of repeating sequence poly(lactic-*co*-glycolic acids) are investigated and the behavior of particular sequences are compared both to each other and to random copolymer controls. A portion of this work has been previously reported.<sup>75</sup>

#### 3.2 INTRODUCTION

PLGA copolymers, being biocompatible and biodegradable, have attracted significant attention for as potential mediators for *in vivo* drug delivery.<sup>2,3,12,13,63,66,97-102</sup> The degradation mechanism for PLGAs is simple hydrolysis; ester bonds are cleaved and the copolymer matrix collapses resulting in the release of encapsulated species.<sup>66,103,104</sup> The products of the copolymer degradation are lactic and glycolic acids which can be metabolized to H<sub>2</sub>O and CO<sub>2</sub> via the Krebs cycle.<sup>105</sup> The facile bioassimilation of these products makes PLGA safe for *in vivo* applications.<sup>99</sup> PLGA can be used in a variety of morphologies: scaffolds,<sup>106</sup> film,<sup>107</sup> fibers,<sup>108</sup> foams,<sup>109</sup> and microparticles.<sup>49,64</sup> Among these different morphologies, microparticles, with a

high loading ability and an excellent penetration into tissues, are considered one of the most promising carriers and are widely used in topical, oral and parenteral drug delivery systems.<sup>5,110-</sup>

113



**Figure 14.** Mechanism for bulk and surface degradation of a PLGA particle. Lighter color indicates water diffusion through particle.

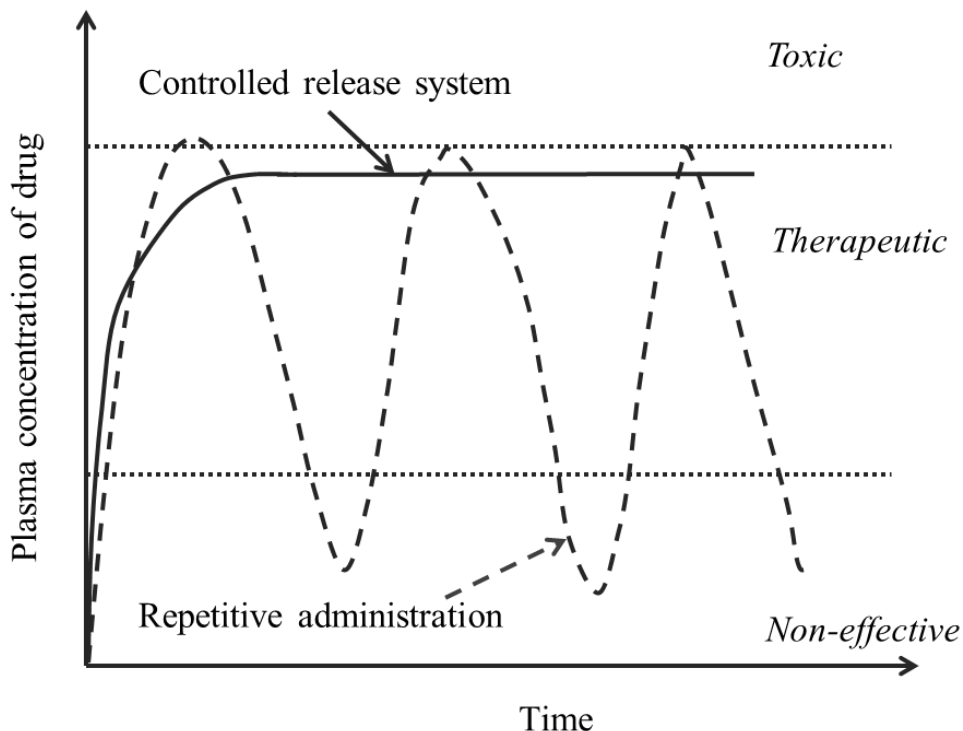
Hydrolytic degradation is a combination of the water uptake and the cleavage of hydrolyzable bonds. The relative rates of these two processes give rise to the two major mechanisms of material degradation, bulk and surface (Figure 14). In bulk degradation water uptake is much faster than bond cleavage and samples are usually wetted throughout before bonds start to cleave. In contrast, sample with a surface degradation show a synchronous uptake of water and cleavage of bonds.<sup>114</sup> For random PLGA, the rate of water uptake is faster than the rate of bond cleavage, so that bulk degradation predominates.<sup>114,115</sup> The degradation mechanism of microparticles during hydrolysis therefore can be considered to be homogeneous,<sup>66</sup> which means the hydrolytic rate of core and surface of particles are nearly equal.<sup>116</sup>

The degradation products, lactic and glycolic acids, can also contribute to the hydrolysis process. If the acid by-products are able to diffuse away from the intact polymer, they have no effect. However, because hydrolysis products cannot rapidly diffuse from inside the microparticles (or other delivery vehicles), the local pH value can decrease dramatically.<sup>117</sup> As ester cleavage is catalyzed by acid, the result is product-stimulated autocatalysis of the degradation.<sup>118</sup> The accumulated acids inside microparticles may also unfavorably affect drug stability.

Autocatalysis is of particular importance in degradation of PLGA. Although it is hard to control autocatalysis during hydrolysis of PLGA, several factors still have effect on degradation behavior, and can be used to control degradation of PLGA devices. These factors include molecular weight,<sup>119</sup> polymer composition,<sup>64</sup> end groups of polymer chain,<sup>120</sup> device size, morphology and porosity,<sup>118,121,122</sup> degradation temperature,<sup>123</sup> and pH value of degradation medium.<sup>124</sup> Of these factors that can effect on the degradation behavior, adjusting intrinsic properties of PLGAs and post-processing of devices have been the focus of most efforts because these factors are not related to the degradation environment. Other factors, such as degradation temperature and pH value of degradation medium, are usually more difficult to control *in vivo*.

Significant research has focused on the determination of the characteristics that control the degradation behavior of PLGA used in devices. Park *et al*,<sup>119</sup> reported that the  $T_g$  of PLGA with a low initial molecular weight will drop faster than that of a polymer with a high initial molecular weight. The ratio of lactic to glycolic units has also been found to be important in degradation. The degradation rate of glycolic-glycolic links in polymer is 1.3 times higher than that of lactic-lactic links.<sup>125</sup> In addition to the steric effects that control the rate of hydrolysis, degradation of polymers with high lactic contents will also be slow because hydrophobic lactic

blocks are more likely to crystallize than glycolic unit under the hydrolysis conditions.<sup>64</sup> Size and morphology of PLGA devices also act important roles in degradation behavior. As autocatalysis happens during degradation, acidic hydrolysis products in smaller sized devices can diffuse out much faster at the early stage of degradation. Fewer acids accumulate inside, therefore reduce the autocatalysis.<sup>118,121,122</sup>



**Figure 15.** Comparison of controlled and traditional drug delivery system, adapted from ref. 112 with permission.<sup>112</sup>

Degradation control of PLGA used as drug delivery carriers can help construct a controlled drug delivery system.<sup>112,126</sup> Traditional drug delivery by tablets or injection usually results in high plasma concentration of drug in therapeutic window for each dose. The effective concentration is maintained by intake of new dose when it drops in the non-effective window (Figure 15). Frequently taking new doses usually leads to a toxic concentration of drug in plasma or termination of therapy because of the non-effective concentration of drug. An ideally controlled drug delivery system should include a fast release of drug to therapeutic concentration

and a sustained release to keep the concentration of drug in plasma follows for a long time period (Figure 15).

PLGA microparticles have been used as carriers in controlled drug delivery systems.<sup>5,110-112,115,126,127</sup> *In vitro* and *in vivo* release behaviors of hydrophilic drugs, lipophilic drugs, protein and DNA have been widely studied. The general drug release mechanism is considered to be 1) erosion of polymer matrix, 2) diffusion of drug molecules from matrix to medium and 3) dissolution of drug in medium. Factors that control release rates including drug properties, the degradation rate of polymer and the porosity of matrix.

PLGA microparticles are usually prepared by several methods: emulsion solvent evaporation,<sup>100</sup> phase separation<sup>128</sup> and spray-drying.<sup>103</sup> The former is more frequently used for the study of drug release rates, because the drugs with either lipophilic or hydrophilic properties or with high molecular weight such as DNA can be encapsulated during the formation of the particles.<sup>5,129</sup> Polyvinyl alcohol solution (PVA) has been employed as commonly used emulsifier because the particles made by PVA emulsifier are uniform and the surface is smooth.<sup>64</sup> The particles size can be modified by changing the speed of homogenizer from nanosize to up to 300  $\mu\text{m}$  to achieve different rate for the drug delivery.<sup>100</sup>

*In vitro* hydrolytic degradation studies are usually carried out in pH = 7.4 buffer under 37 °C to emulate the conditions in the body. Hydrolysis rates are monitored by the decrease of molecular weight by size exclusion chromatography (SEC), increase of lactic acid in the solution by high performance liquid chromatography (HPLC) or an enzymatic method, and change of microparticles morphology by scanning electron microscopy (SEM).<sup>64</sup> Thermal property changes which reflect changes in polymer morphology during degradation are determined by differential scanning calorimeter (DSC).

As PLGAs with controlled sequence are found to have sequence-specific effects on the NMR spectroscopy, solution phase conformation and thermal properties,<sup>48</sup> they are potential to have sequence-specific bulk properties. In this chapter, the effect of monomer sequence on hydrolytic degradation behavior of repeating sequenced PLGAs will be discussed in detail. The effect on the encapsulation and release of a guest molecule—rhodamine-B, will also be explored.

### **3.3 EXPERIMENTAL**

#### **3.3.1 Size exclusion chromatography (SEC)**

Molecular weight data were determined by SEC using a Waters 515 HPLC pump with phenogel 10<sup>4</sup> and 500 Å columns and a Waters 2414 refractive index detector. THF was used as the mobile phase of the SEC with a flow rate of 0.5 ml/min. The sample was dissolved in THF, filtered and then injected into a 20 µL loop. The number and weight average molecular weight were determined from the SEC curve by calibration with polystyrene standards.

#### **3.3.2 Differential scanning calorimetry (DSC)**

The thermal properties of the microparticles were determined by DSC measurements. Microparticle samples (~ 5 mg) were placed in aluminum pans and analyzed with a Perkin-Elmer DSC 6 instrument by scanning from 10 °C to 200 °C with a heating rate of 10 °C / min. Reported transitions were obtained from the first heating cycle.



### **3.3.3 Fluorescence spectroscopy (FL)**

The concentration of rhodamine-B released from microparticles to phosphate buffer was determined by FL using SpectraMax M2 Microplate Readers. Supernatant (250  $\mu$ L) was added to a 96-well plate and the emission ( $\lambda_{\text{max}} = 577$  nm) was recorded by excitation  $\lambda_{\text{ex}} = 556$  nm.

### **3.3.4 UV-Vis absorbance spectroscopy**

Absorbance of RhB dissolved in acetonitrile was determined by Perkin Elmer UV-Vis spectrometer. The scanning wavelength was from 700 nm to 300 nm and the scan rate was 60 nm/min. The maximum absorbance was recorded at  $\lambda_{\text{max}} = 556$  nm.

### **3.3.5 Particle size determination**

Microparticles sizes were determined by light scattering using a ZetaPALS instrument (Brookhaven Instrument Corporation). An ultrasonicator was used to give well-dispersed suspension of microparticles (5mg) in water (15 ml).

### **3.3.6 Preparation of microparticles**

Microparticles of all PLGAs were prepared by a standard emulsion method.<sup>5</sup> A solution of PLGA polymer (0.2 g) in  $\text{CH}_2\text{Cl}_2$  (4 ml) was emulsified in a poly(vinyl alcohol) (PVA) solution (60 ml, 2 % w/v) using a homogenizer (10,000 rpm) for 1 min. The emulsion was then poured into another PVA solution (80 ml, 1% w/v) and stirred for 3 h at room temperature to evaporate

the methylene chloride. The microparticles were centrifuged and washed with deionized water 3 times and freeze-dried overnight to remove the residual solvent. The microparticles were stored in a desiccator.

### **3.3.7 *In vitro* hydrolysis study**

Microparticles for each polymer (20 mg) were dispersed in phosphate buffer solution (1 ml, pH = 7.4) in multiple microcentrifuge tubes (2 redundant samples for each time period). All tubes were incubated at 37 °C. The buffer was exchanged every two days by centrifugation followed by the decanting of the supernatant. At designated intervals, all microparticles from an individual microcentrifuge tube were collected, washed and freeze-dried. All data reported are the averages of the measurement for the two redundant samples.

### **3.3.8 Lactic acid release study**

The lactic acid released from PLGA microparticles to the media during *in vitro* hydrolytic degradation was determined by the enzymatic analysis. Generally, during changing the buffer, the supernatant was collected and kept at -20 °C. The supernatants was then thawed and neutralized to convert L-lactic acid to L-lactate. The L-Lactate was then determined using Lactate biosensor instrument (Yellow Spring Inc. OH). Concentration of lactic acid was calculated based on the known ratio of L-lactic acid units to glycolic units in the polymer. In the case of racemic copolymers, the measured L-lactic acid concentration was doubled to reflect total lactic acid released.

### **3.3.9 Encapsulation of rhodamine-B in PLGA microparticles**

The encapsulation of rhodamine-B (RhB) into PLGA microparticles was achieved using the double emulsion method.<sup>5,111</sup> To avoid photo bleaching of RhB, all the containers were covered with aluminum foil during preparation. RhB aqueous solution (200  $\mu$ L) was added to the solution of polymer in  $\text{CH}_2\text{Cl}_2$  (4 ml, 5% w/v) and mixed for 10 sec using ultrasonicator (25% power). The mixture was emulsified in a poly(vinyl alcohol) (PVA) solution (60 ml, 2 % w/v) using a homogenizer (10,000 rpm) for 1 min. The emulsion was then poured into another PVA solution (80 ml, 1% w/v) and stirred for 3 h at room temperature to evaporate the methylene chloride. The microparticles were centrifuged and washed with deionized water 3x and freeze-dried overnight to remove the residual solvent. The microparticles were stored in a desiccator before being used.

### **3.3.10 Determination of loading yield of RhB in PLGA microparticles**

Microparticles (10mg) with RhB were dispersed in acetonitrile (1 mL) in a glass vial covered with aluminum foil. The dispersion was stirred using a vortex mixer and then ultrasonicated for 30 min. The microparticles completely dissolved during this process and the RhB was dispersed. The concentration of RhB in each sample was determined by comparison with a calibration curve based on the absorption maximum ( $\lambda_{\text{max}} = 556 \text{ nm}$ ).

### **3.3.11 Determination of RhB released from PLGA microparticles**

Microparticles (10 mg) with encapsulated RhB were dispersed in phosphate buffer solution (PBS, pH 7.4, 0.01M, 1 mL) in microcentrifuge tubes (4 redundant samples for each

measurement). The centrifuge tube was covered with aluminum foil and dispersed by vortex for 5 min. The samples were placed in a rotating mixer (8 rpm) in a 37 °C incubator. Individual tubes were collected and centrifuged daily during the first 10 days. The supernatants were collected for spectroscopic analysis. The microcentrifuge tubes were refilled with fresh PBS (1 mL) and returned to the incubator to continue the release experiment. After 10 days, the interval between sample collections was extended to every 2 days. After 20 days samples were collected every 3 days. All data reported are averages of for the four redundant samples.

For calibration, a series of standard solutions of RhB in PBS were stored under the same conditions as the release studies. The RhB concentrations in the supernatants solutions collected as described were measured by comparison with these standards. The fluorescence emission at  $\lambda_{\text{max}} = 577 \text{ nm}$ , excited by  $\lambda_{\text{ex}} = 556 \text{ nm}$ , was used as the basis for the measurement.

### 3.4 RESULTS AND DISCUSSION

#### 3.4.1 Naming convention

The L-lactic unit, *rac*-lactic unit and glycolic unit are abbreviated as **L**, **L<sub>rac</sub>** and **G**, respectively. Repeating sequenced PLGA copolymers prepared by SAP are named by listing the order of segmer sequences from the C-side to the O-side preceded by **Poly**. Therefore, **Poly L<sub>rac</sub>LG** is the polymer prepared from segmer with sequence of *rac*-lactic acid, L-lactic acid and glycolic acid by using SAP polymerization method. The random PLGAs are named with initial **R** followed by the preparation method (SAP or ROP) and the lactic unit ratio. Thus, the **R-SAP 50** is the

random PLGA prepared by SAP with 50% of **L** unit. **R-ROP 50** and **R-ROP 75** which were used as the controls, are two commercial PLGAs named based on the lactic unit ratio.

### 3.4.2 Polymers

A series of PLGA RSCs and one random copolymer, **R-SAP**, were prepared as described previously using the SAP methodology (See Chapter 1).<sup>48,75,81</sup> Characterization data for these polymers and two commercially purchased random copolymers prepared using a ROP method, **R-ROP 50** and **R-ROP 75**, are summarized in Table 5. Molecular weights were determined by size exclusion chromatography (SEC) in THF and are reported relative to polystyrene standards. Particle sizes were determined by dynamic light scattering.

**Table 5.** PLGAs used for the hydrolysis and lactic acid release studies

	$M_n^a$ (kDa)	$M_w^a$ (kDa)	PDI <sup>a</sup>	Particle size <sup>b</sup> ( $\mu\text{m}$ )	L : G
<b>R-ROP 50</b>	32	43	1.3	2.1	1:1
<b>R-SAP 50</b>	31	40	1.3	4.0	1:1
<b>R-ROP 75</b>	55	66	1.2	2.8	3:1
<b>Poly LG(26k)</b>	26	35	1.3	1.8	1:1
<b>Poly LG(16k)</b>	16	25	1.6	2.3	1:1
<b>Poly L<sub>rac</sub>G</b>	49	103	2.1	3.5	1:1
<b>Poly LLG</b>	33	54	1.6	2.2	2:1
<b>Poly L<sub>rac</sub>LG</b>	35	46	1.3	5.0	2:1
<b>Poly GLG</b>	16	22	1.3	2.4	1:2

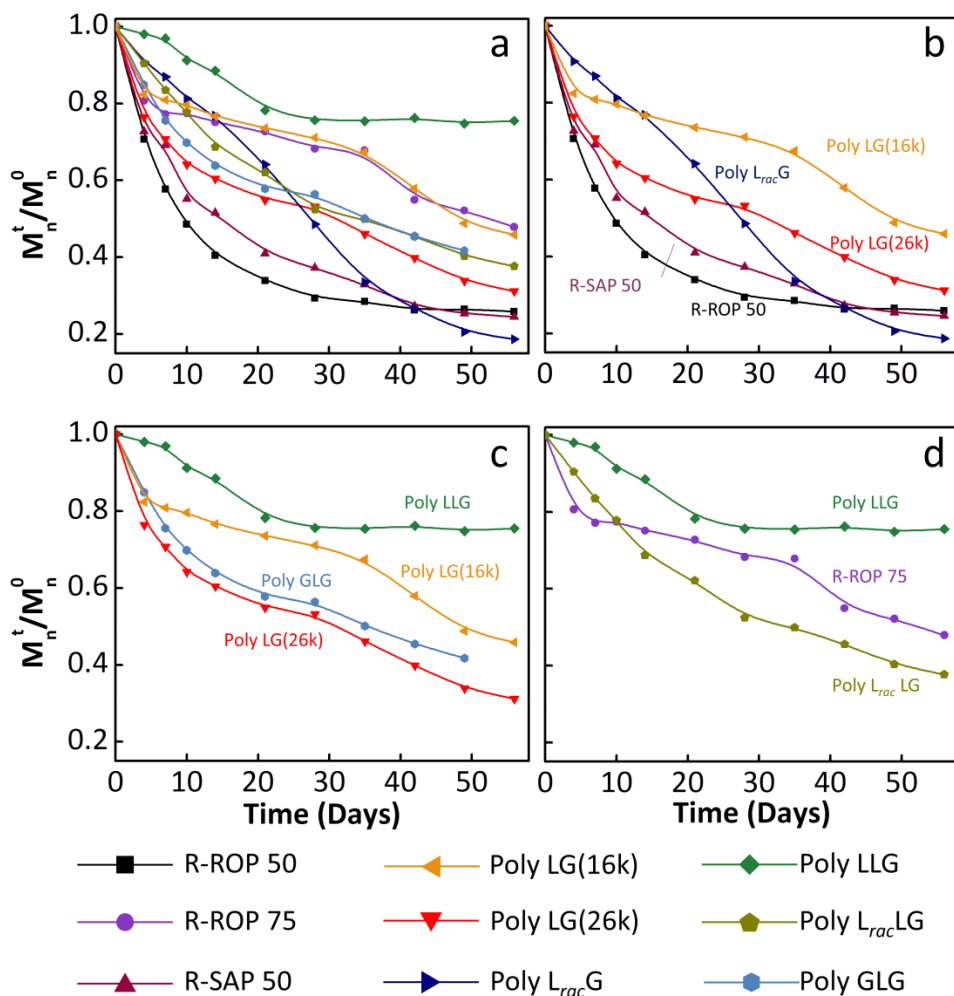
<sup>a</sup>Molecular weights and polydispersity indices determined by SEC in THF vs. polystyrene standards <sup>b</sup>Particle sizes determined by dynamic light scattering

### 3.4.3 Hydrolysis Profiles

To correlate the dependence of hydrolytic degradation behavior on the sequence of PLGA copolymers, a series of sequenced PLGAs and three random PLGAs were selected for an *in vitro* hydrolytic degradation study. As it is common to use PLGA microparticles as hosts for drug delivery and many hydrolysis studies have been conducted on the random PLGA copolymers formulated thusly,<sup>110</sup> we chose in these initial studies to monitor the hydrolysis behavior of the PLGA RSCs using this widely practiced protocol. The copolymers were formulated into microparticles with sizes ranging from 2 to 5  $\mu\text{m}$  using a standard emulsion method.<sup>5</sup> This narrow particle size range was purposely targeted to minimize the impact of size-dependent autocatalysis on PLGA degradation.<sup>64,118</sup> Microparticles of each polymer were divided into multiple parallel reaction vessels and suspended in a phosphate buffer (pH 7.4, 37 °C). The supernatant liquid in each was exchanged every two days and retained for analysis of lactic acid content (*vide supra*). The contents of individual reaction vessels were harvested periodically, over the course of 8 weeks, and analyzed by SEC. Selected samples were also characterized by DSC.

The molecular weight profiles for all polymers in this study, normalized relative to the original  $M_n$  for each sample are plotted in Figure 16. for the majority of the samples, there was an initial molecular weight loss that is dramatic relative to the mid-cycle degradation behavior. This initial loss appeared to correlate primarily with the “wetting” of the freeze-dried particles during their first few days in the buffer solution. In this initial phase the shedding of surface coatings that are not well adhered and/or rapid cleavage of surface bonds that are particularly accessible is expected.<sup>121</sup> Consistent with this analysis is the fact that initial rapid loss of weight was seen for both the sequenced and random copolymers. The degree of weight loss in this initial

phase appeared to depend in a complex fashion on sequence, polymer molecular weight, and particle size. For polymers that have either very rapid or very slow hydrolysis rates, this effect was masked or minimized, respectively.

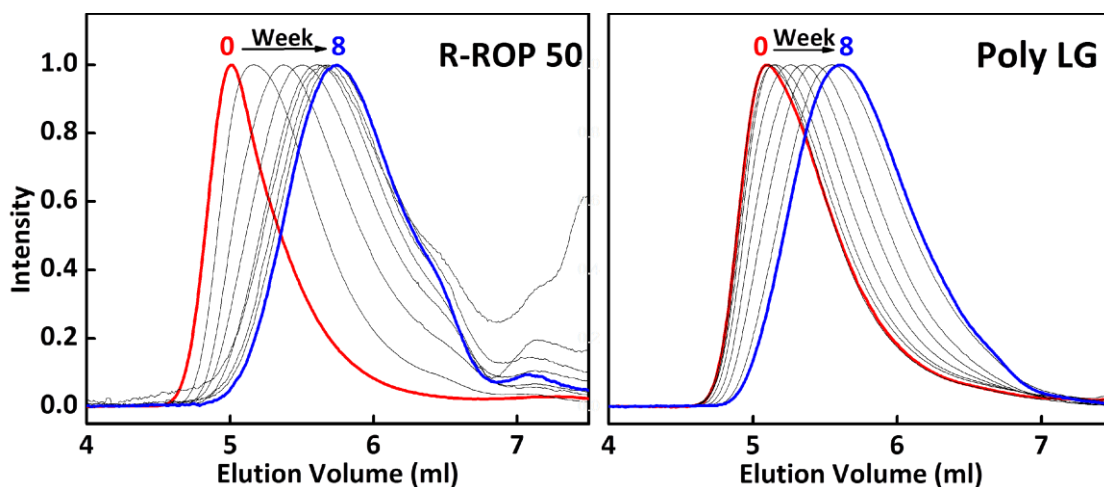


**Figure 16.** Molecular weight loss as a function of hydrolytic degradation time for the repeating sequenced and random sequenced PLGAs (a) plots of all polymers; (b) Comparison of all polymers with a 50:50 LG ratio; (c) Comparison of polymers with varying L:G ratios; (d) Comparison of LLG polymers with varying stereochemistry.

The rate of hydrolysis, after the initial weight loss, did not seem to depend significantly on the initial molecular weight. In Figure 16 the hydrolysis profiles of all samples with a 1:1 L:G are plotted. For **Poly LG(26k)**, it can be seen that after an initial weight loss of 35%, the rate of

weight loss decreases and remains nearly constant to the end of the experiment. **Poly LG(16k)** exhibits a very similar profile: an initial weight loss of 20% followed by more gradual decrease as a function of time. While there is a difference in the relative weight losses at the beginning the rate after the initial loss appears to be relatively independent of the starting molecular weight.

The *most important trend* that can be observed in the hydrolysis studies is that the sequenced copolymers degrade more slowly and at a more constant rate relative to the random copolymers with the same L:G composition. The 1:1 L:G random copolymers, **R-ROP 50** and **R-SAP 50**, both have exponential weight loss profiles, as shown previously.<sup>130</sup> Interestingly, the **R-ROP 50** ( $M_n$  half-life = 10 days) copolymer degraded more quickly than the **R-SAP 50** copolymer ( $M_n$  half-life = 14 days). This difference can be attributed to both the lack of controlled stereochemistry of the racemic **R-ROP 50** copolymer and to differences in microstructure since the **R-ROP 50** copolymer, which was prepared by ring-opening of a mixture of lactide and glycolide monomers, is more blocky in architecture than the **R-SAP 50** copolymer.<sup>40,75</sup> Rapid initial degradation periods were not observed for either of these random copolymers because they were masked by the relatively high rate of hydrolysis.



**Figure 17.** SEC traces for random copolymer (left) and alternating sequence PLGA copolymers (right) with 1:1 ratios of lactic and glycolic acids.



The distinctive differences in degradation between the sequenced and random copolymers can also be observed in the shapes of the SEC traces from which the MW data were extracted. As seen in Figure 17, for example, the molecular weight profile of the random copolymer broadened and became distinctly polymodal over the course of the degradation, while the profile of the sequenced **Poly LG** exhibited only a small amount of broadening. All sequenced copolymers exhibited a similar homogeneity in their evolving SEC traces.

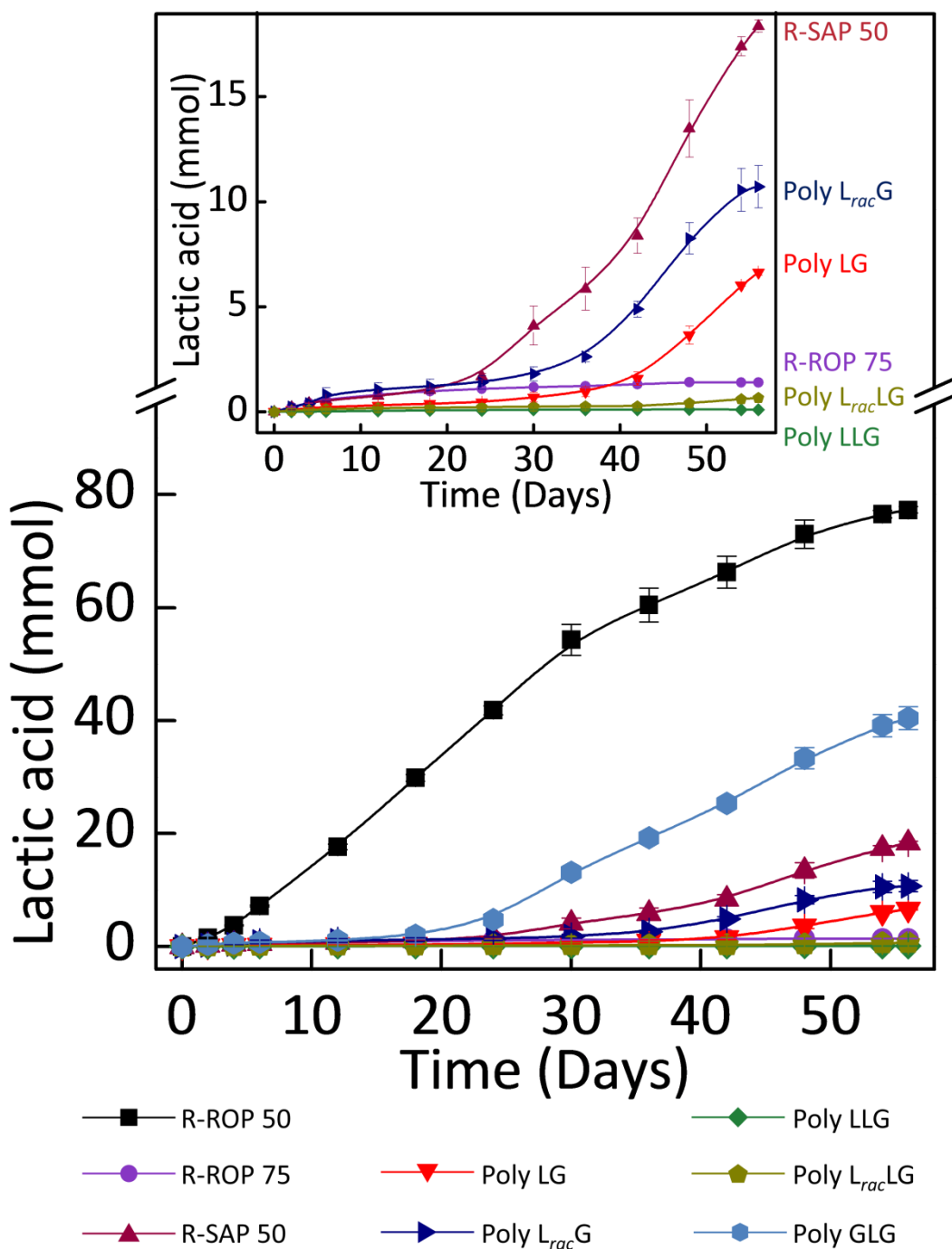
We also examined the relationship of degradation rate to the ratio of L:G in the polymers. It is well-established for random copolymers, that the degradation rate depends on the L:G ratio; high lactic unit content leads to slower hydrolysis rates.<sup>131</sup> Examining the subset of hydrolysis profiles plotted in Figure 16c, it can be seen that for the random controls, **R-ROP 50** and **R-ROP 75** this trend holds. Their  $M_n$  half-lives are 10 and 56 days, respectively. The sequenced copolymers also conform to this trend: **Poly GLG** > **Poly LG(16k)** > **Poly LLG**. The comparison of the **Poly GLG** to **Poly LG(16k)** is used because the **Poly GLG** sample studied had a similarly modest molecular weight.

Finally, the importance of stereosequence can be seen in the hydrolysis behavior of the sequenced copolymers. Hydrolysis was significantly faster for the racemic analogs of the 1:1 and 2:1 L:G polymers, **Poly L<sub>rac</sub>G** and **Poly L<sub>rac</sub>LG**, relative to their stereopure analogs (Figure 16b and d).

#### 3.4.4 Lactic acid release

The degradation rate of the PLGA microparticles was also studied by monitoring the release of lactic acid over time and the sequence dependence of the degradation can be clearly seen in these data. Monitoring of the monomer release yielded a degradation profile that is

complementary to that obtained by the analysis of the molecular weights as discussed in the previous section. The release of L-lactic acid into the buffer solution was assayed using an



**Figure 18.** Lactic acid release rate as a function of hydrolytic degradation time. Inset is the magnified plots of PLGAs with lower lactic acid release rate.

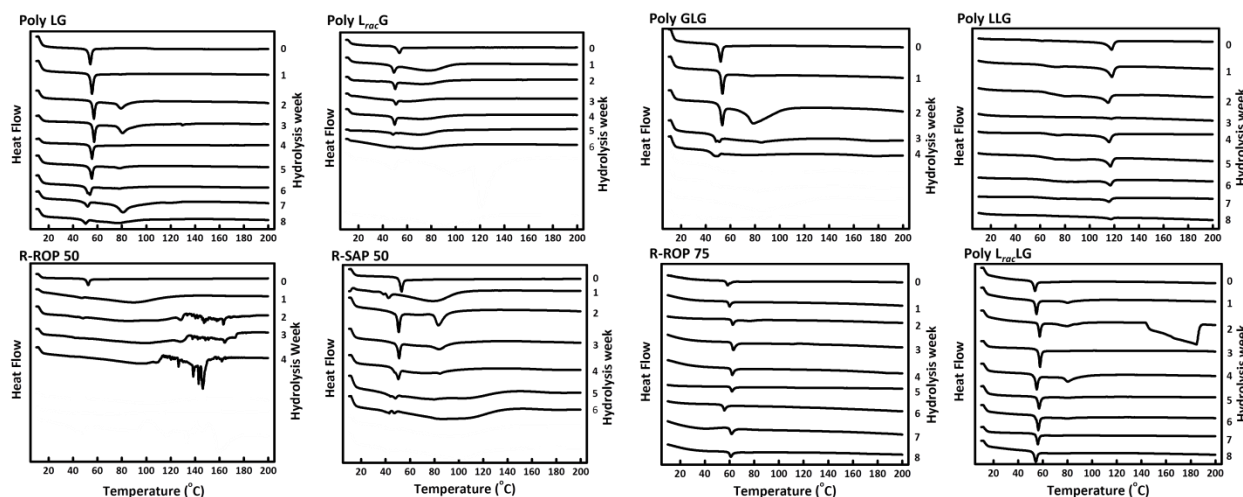
enzymatic method..<sup>132,133</sup> It should be emphasized that the assay reports only monomer and is unresponsive to oligomeric species.

Cumulative release of lactic acid release as a function of time for both the sequenced and random copolymers is plotted in Figure 18. Consistent with the observed rapid degradation of molecular weight discussed above, **R-ROP 50** exhibited the most rapid release of lactic acid. All other samples were significantly slower, including the other 1:1 L:G random copolymer, **R-SAP 50**, and all except the **R-ROP 50** exhibited a profile characterized by an initial quick release of a small amount of the lactic monomer followed by an extended period during which little free lactic acid release was detected. The initial release is likely related to either the wetting of the particle and subsequent dissolution of lightly trapped monomer or the rapid hydrolysis of very short oligomers on or near the surface of the particle. The low release period that followed is not a dormant period, as we know from the molecular weight data, but rather corresponds to a time when the polymers were partly hydrolyzed to oligomers that do not register in the enzymatic assay. Only in the later stages of hydrolysis did these oligomers degrade enough to release monomeric lactic acid.

Significant sequence-dependent trends were observed in these data and most corresponded well with those observed in the molecular weight studies. In particular, for the 1:1 LG polymers the release rate followed the following trend: **R-ROP 50**  $\gg$  **R-SAP 50**  $>$  **Poly LG** (Figure 18, see inset for expansion of the profiles for the more slowly degrading polymers). The random copolymer, **R-ROP 50**, was much faster than the less blocky **R-SAP 50** and both were faster than the stereopure alternating **Poly LG**. There was also a pronounced dependence of lactic acid release rate on L:G ratio, with a trend, **Poly GLG**  $\gg$  **Poly LG**  $>$  **Poly LLG**, that runs contrary to what would be expected based on the total L content (given that this is an assay for

L). The differences in total lactic acid release are significant over the time period studied: 40 mmole from **Poly GLG** vs. 0.1 mmol for **Poly LLG**. Finally, the rate of release of lactic acid was faster for the racemic sequenced copolymers **Poly L<sub>rac</sub>G** > **Poly LG** and **Poly L<sub>rac</sub>LG** > **Poly LLG**.

### 3.4.5 Thermal properties



**Figure 19.** DSC thermograms of PLGAs in the hydrolysis study.

The unique degradation behaviors of the sequenced PLGAs relative to random PLGAs were further characterized through the study of their thermal behavior. DSC thermograms for selected PLGAs are presented as a function of degradation time in Figure 6. The first heating cycle is reported to reflect the *in situ* thermal properties of the microparticles after hydrolysis. The DSCs of the polymers that have a 1:1 L:G ratio, showed a single phase transition,  $T_g \cong 50^\circ\text{C}$ , at the beginning of the experiment. The most dramatic difference can be seen in the comparison of the thermograms for **Poly LG(26k)** and **R-ROP 50**. The random sample degraded so quickly that reliable DSC data could not be acquired after week 4, while those of **Poly LG(26k)** continued to exhibit clear transitions. There was also a distinctive difference in the transitions exhibited. The

DSC trace for **Poly LG(26k)** appeared nearly the same in week 8 as it did prior to hydrolysis. The  $T_g$  shifted slightly to lower temperature and broadened consistent with the drop in molecular weight and there was a new broad peak at ca. 80 °C that is likely due to the melting of small amounts of crystalline oligomers.<sup>75</sup> In contrast, a clear  $T_g$  for **R-ROP 50**, was no longer visible by the end of week 1 and the DSC traces are dominated by multiple melting transitions ranging from 80 to 160 °C that have been shown in prior studies to be due to crystallized oligomers with a high lactic acid content.<sup>64</sup>

**Poly L<sub>rac</sub>G**, although faster to degrade, followed the same pattern as **Poly LG**, exhibiting a clean but slightly shifted  $T_g$  until the samples could no longer be analyzed due to low molecular weight. The other random copolymer, **R-SAP 50** exhibited a behavior intermediate between the **Poly LG** and **R-ROP 50** polymers as would be expected from its less blocky nature. The  $T_g$  of the bulk remained distinct but a broad melting transition which is lower in temperature than that of **R-ROP 50** dominated after week 5. **Poly GLG**, which has a slightly lower  $T_g$  of ca. 52 °C, exhibited the same uniformity of degradation as that seen for the sequenced LG polymers.

The DSCs for the higher L:G content polymers showed that the RSC **Poly LLG** was semi-crystalline with a  $T_g$  of 60 °C and  $T_m$  of 118 °C. **Poly L<sub>rac</sub>LG** and **R-ROP** in contrast, exhibited only  $T_g$  transitions of 53 and 58 °C, respectively. None of these polymers changed significantly over the time period of the experiment, however, since their degradation is quite slow.

### 3.4.6 Rhodamine-B release

As one of the primary applications of PLGA is drug delivery,<sup>134</sup> we have examined the effect of sequence on the release rate for microparticles loaded with Rhodamine-B (RhB), a low molecular weight hydrophilic dye used as a model in *in vitro* drug-delivery studies because of its water solubility and characteristic absorbance at 556 nm.<sup>135</sup> RhB was incorporated into microparticles via a double emulsion method.<sup>5</sup> RhB loading was estimated by dissolving a weighed portion of each sample in a known volume of acetonitrile and comparing the UV-Vis absorption with a calibration curve created from solutions of RhB of known concentration. This method gave more precise and reproducible results than a low pH digestion of the particles in water<sup>127</sup> as we observed that the absorption intensity of acidic RhB solutions decreased rapidly with time. The polymers selected for this study were **Poly LG**, **Poly L<sub>rac</sub>G**, and **R-ROP 50** (Table 6, top section). These polymers had comparable but higher molecular weights than those used in the hydrolysis studies but were consistent relative to each other.

To study the relative loading capacities of the sequenced copolymer and plan for subsequent *in vitro* release studies, the **Poly LG** particles were prepared with different initial RhB concentrations. Specifically, the concentration of the RhB solution was adjusted from 0.2 to 1.0 mg/ml. The release of RhB from the resulting particles was analyzed over a period of 15 days and the data are plotted in Figure 20. These data were derived from a set of samples prepared and handled under identical conditions to ensure that any effect from photobleaching was systematic and did not affect the following comparisons. The random copolymer reached its maximum cumulative release of approximately 90% after only 9 days while all of the sequenced samples released their payload at a lower rate. Of particular interest, however, was the dramatic difference in loading efficiency for the two types of polymers. When identical amounts of RhB

were used in the particle formulation/loading procedure (0.2 mg), the random copolymer particles encapsulate 40% of RhB whereas the alternating copolymer **Poly LG-RhB1** exhibited a loading efficiency of 19%. Much larger concentrations of RhB were necessary to attain loadings of the dye into **Poly LG** that were comparable to those achieved in the random copolymer.

**Table 6.** PLGA polymer and loading properties

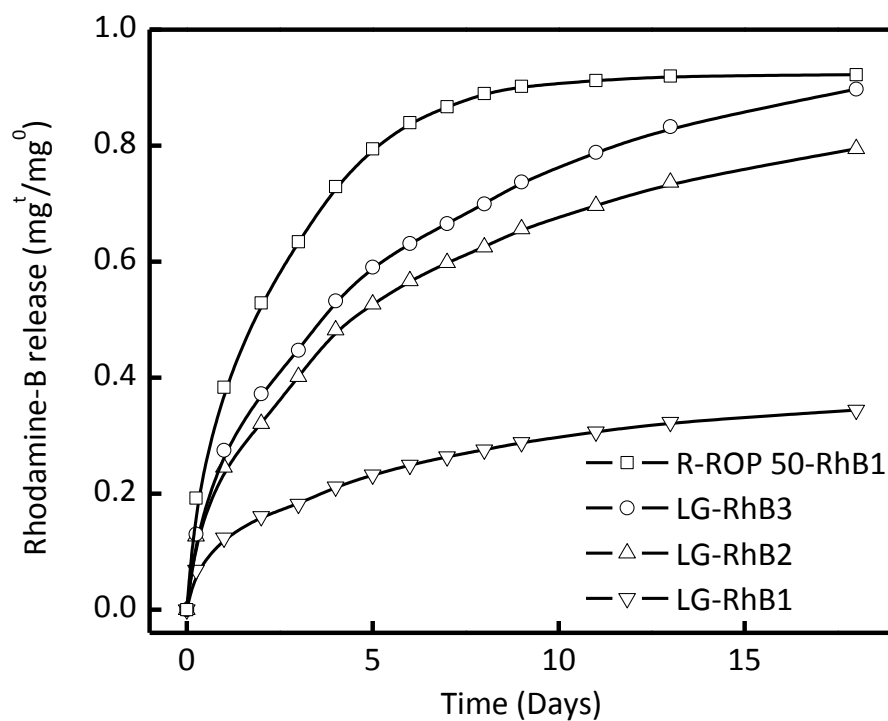
Polymer	M <sub>n</sub> <sup>a</sup> (kDa)	PDI <sup>a</sup>	RhB (mg)	Load (mg x 10 <sup>-4</sup> / mg)	Loading rate <sup>b</sup> (%)
<b>LG-RhB1</b>	37.2	1.4	0.2	1.9	19
<b>LG-RhB2</b>	37.2	1.2	0.6	2.2	7.5
<b>LG-RhB3</b>	37.2	1.4	1.0	3.4	5.9
<b>R-ROP 50-RhB1</b>	32.0	1.3	0.2	4.0	40
<b>LG-RhB4</b>	37.2	1.4	1.0	2.7	5.4
<b>L<sub>rac</sub>G-RhB</b>	38.2	1.4	1.0	2.8	5.6
<b>R-ROP 50-RhB2</b>	32.0	1.3	0.1	2.9	58
<b>R-ROP 50-RhB3</b>	32.0	1.3	1.0	5.9	12

<sup>a</sup>Molecular weights and polydispersity indices determined by SEC in THF vs. polystyrene standards. <sup>b</sup>Based on 200 mg polymer sample size, calculated according to Equation 2.

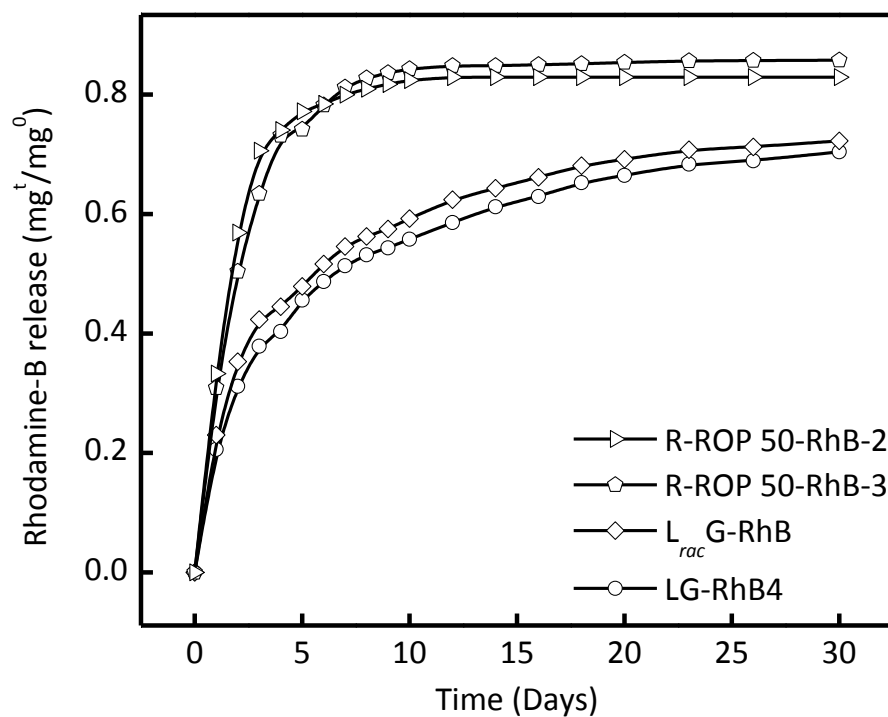
**Equation 2**

$$Loading\ rate = \frac{Mass_{RhB\ loaded}}{Mass_{RhB\ in\ total}} \times 100\%$$

Based on the data collected from the loading efficiency experiments microparticles of both sequenced and random copolymers with the same RhB loading were produced. A new study of the release rates was conducted and the results are plotted in Figure 21. Poly LG, Poly L<sub>rac</sub>G, and the random copolymer, R-ROP 50-RhB2, all have similar loadings while R-ROP 50-RhB3 was prepared with a much higher RhB load.



**Figure 20.** Release of Rhodamine-B from PLGA microparticles immersed in a pH 7.4 buffer at 37 °C: 15 day study focusing on particle loading.

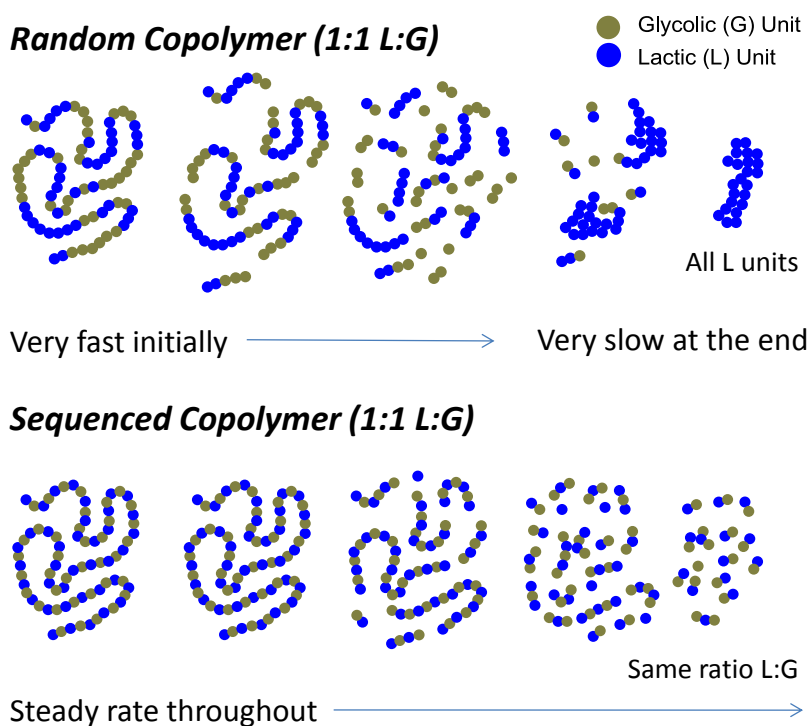


**Figure 21.** Release of Rhodamine-B from PLGA microparticles immersed in a pH 7.4 buffer at 37 °C: 30 day study focusing on the effect of sequence.



The key result from this study is that RhB release was significantly more gradual for both of the sequenced copolymers when compared to the random copolymers. This trend held despite significant differences in the **Poly L<sub>rac</sub>G** and **Poly LG** degradation rates between days 0 and 20 (*vide supra*). Additionally, random copolymers gave nearly the same release profile despite the differences in loading, in contrast to the behavior of **Poly LG** in Figure 20. Along with the differences in loading efficiency (Table 6), these results suggest that RhB release from sequenced copolymer particles depends not only on hydrolysis rate, but also on other factors.

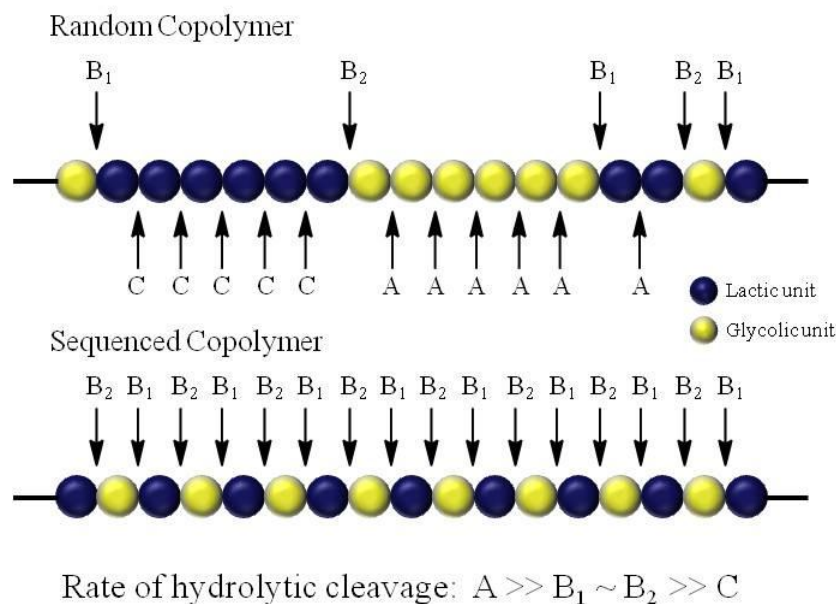
### 3.4.7 Mechanism discussion



**Figure 22.** Proposed difference in hydrolysis pattern for random and sequenced PLGA copolymers with the same L:G ratio

The introduction of sequence control to the PLGA system changes the hydrolysis pattern significantly relative to random analogues. Both molecular weight loss and lactic acid release

measurements establish that the sequenced copolymers degrade at a steady rate which contrasts with the rapid, exponential profile exhibited by the random copolymers with similar L:G ratios. The differences are likely attributable to the homogeneity of the sequenced copolymers. It has been observed by others who have studied the degradation of random PLGA copolymers that hydrolysis of the more sterically accessible glycolic units is rapid relative to lactic-rich blocks.<sup>125</sup> In Figure 22 a conceptual comparison is made between the simple alternating copolymer **Poly LG** and **R-ROP 50**. The sequenced copolymer breaks down evenly as hydrolysis proceeds, while the random copolymer can be selectively attacked in such a way that slowly degrading lactic oligomers are left to crystallize. The uniformity of the cleavage sites in polymer chains is a key reason for the gradual and controllable degradation of **Poly LG**. The RSC presents only two types of hydrolysis sites (Figure 23). Nucleophilic attack by water at the glycolic acid carbonyl breaks the C-O bond to the adjacent lactic acid (B<sub>1</sub>) while attack at the lactic acid carbonyl should cleave the adjacent glycolic acid (B<sub>2</sub>). In contrast, the random copolymers have a wide variety of sites that would be expected to exhibit a more diverse range of reactivity rates with water. At the simplest level one would suspect that G-G connections will cleave more quickly than G-L/L-G which should cleave more quickly than the hydrophobic L-L connections. Data on the hydrolysis of random PLGAs is consistent with this hierarchy of rates. Both the SEC and DSC data for the 1:1 copolymer are consistent with this model.



**Figure 23.** Illustration of the distinct hydrolytic degradation sites in random and alternating PLGA copolymers

The specific characteristics of the degradation profiles for the sequenced copolymers are also of interest. In contrast to the exponential degradation observed for the random copolymers, the sequenced copolymer profiles have a somewhat sigmoidal shape. Based on our experimental observations, we believe that the profile can be attributed, in part, to the following phases of particle degradation. The initial steep rate of hydrolysis can be attributed the “wetting effect” discussed earlier. The middle region, by this hypothesis represents the sequence dependent degradation rate while the final steeper curve is due to particle collapse effects including the solubility of the increasingly short oligomers and the increase in surface area caused by the physical disintegration of the particle. As no simple fitting algorithm captures this complexity, and as there are likely other factors that contribute to the degradation, a more quantitative assignment of rates cannot be made at this time. Additional experiments and modeling studies, which have yet to be undertaken for ordered copolymer systems of this type and which can account for a population of polymer chains,<sup>136</sup> will be required before these degradation profiles can be fully explained and the rates quantified.

The RhB release studies presented suggest that the hydrolysis rate profiles and release rates correlate to some degree. The sequenced copolymers degrade at a slower rate than do random copolymers and also release the encapsulated dye molecule more gradually. This is a promising discovery as many drug delivery applications specifically target a slow release over time. It is clear, however, from the loading capacity studies and early period release data that the slower release rate depends on more than degradation rate. The repeated **LG** sequence may, for example, strengthen electrostatic or hydrophobic interactions between the guest and polymer, which would also slow the rate of release.

It is also of interest to compare the hydrolysis and release behavior of our SAP-produced copolymer with that previously reported by Dong *et al.* for a ROP-produced alternating copolymer. There are both similarities and differences in the two systems.<sup>137</sup> Although Dong *et al.* did not directly compare their polymer with a random control when studying hydrolysis rate, the plot of molecular weight vs. time for their microparticles shows a nearly linear decrease analogous to our observations. Dong *et al.* also studied release profiles from their alternating copolymer, although their guest was bovine serum albumin (BSA) which is a large protein, as compared to the small molecule release agent used in the current work. For this part of the study a random copolymer control was used. Similar to our RhB studies, they observed a higher burst release from the random copolymer than from the ROP-alternating copolymer in the first few days. It is difficult to compare the systems beyond this point, however, as the model protein BSA was released very slowly relative to RhB, reaching only 20% completion from the random copolymer within 40 days and less than 10% from the ROP-alternating copolymer. Another difference which is likely related to the particular characteristics of BSA vs. RhB, was the fact

that they reported a similar (and much higher, > 30%) loading efficiency for both the random and the ROP-alternating copolymer.

The potential implications of the observed degradation and release behavior of the sequenced PLGAs are clearly relevant to the biomedical applications that employ these materials as 1) simply ordering L:G units in a repeating sequence leads to more sustained release of encapsulated guests as compared with a random copolymer with the same overall composition; 2) a slower loss of molecular weight should lead to longer retention of mechanical properties which is important in stem cell scaffolding applications; and 3) a more homogeneous degradation profile may lead to more uniform erosion or clearance of the polymer matrix preventing the accumulation of extremely slow-degrading material, such as lactic acid oligomer crystals, that cause local inflammation long after the function of the PLGA construct is completed.<sup>138</sup> Future studies will probe these questions in greater detail.

### **3.4.8 RhB encapsulation and release in Poly L<sub>2</sub>G<sub>2</sub> and Poly L<sub>3</sub>G<sub>3</sub>**

In this section the encapsulation and release behavior of RhB of two other sequenced PLGAs are described. These results have not been included in the previous discussion due to the strangely atypical behavior of one or both of these samples. The behavior was sufficiently anomalous that we feel that the studies of these polymers must be repeated with appropriate controls.

The PLGAs are **Poly GLLG (Poly L<sub>2</sub>G<sub>2</sub>)** and **Poly GLLLGG (Poly L<sub>3</sub>G<sub>3</sub>)**. The polymer properties are listed in

Table 7 along with those of **LG-RhB4** for comparison.

**Table 7.** PLGA polymer and loading properties

Polymer	M <sub>n</sub> <sup>a</sup> (kDa)	PDI <sup>a</sup>	RhB (mg)	Load <sup>b</sup> (mg x 10 <sup>-4</sup> /mg)	Loading rate (%)
<b>L<sub>2</sub>G<sub>2</sub>-RhB</b>	35.0	1.2	1.0	2.4	4.8
<b>L<sub>3</sub>G<sub>3</sub>-RhB</b>	45.9	1.2	1.0	1.6	3.2
<b>LG-RhB4</b>	37.2	1.4	1.0	2.7	5.4
<b>R-ROP 50-RhB2</b>	32.0	1.3	0.1	2.9	58

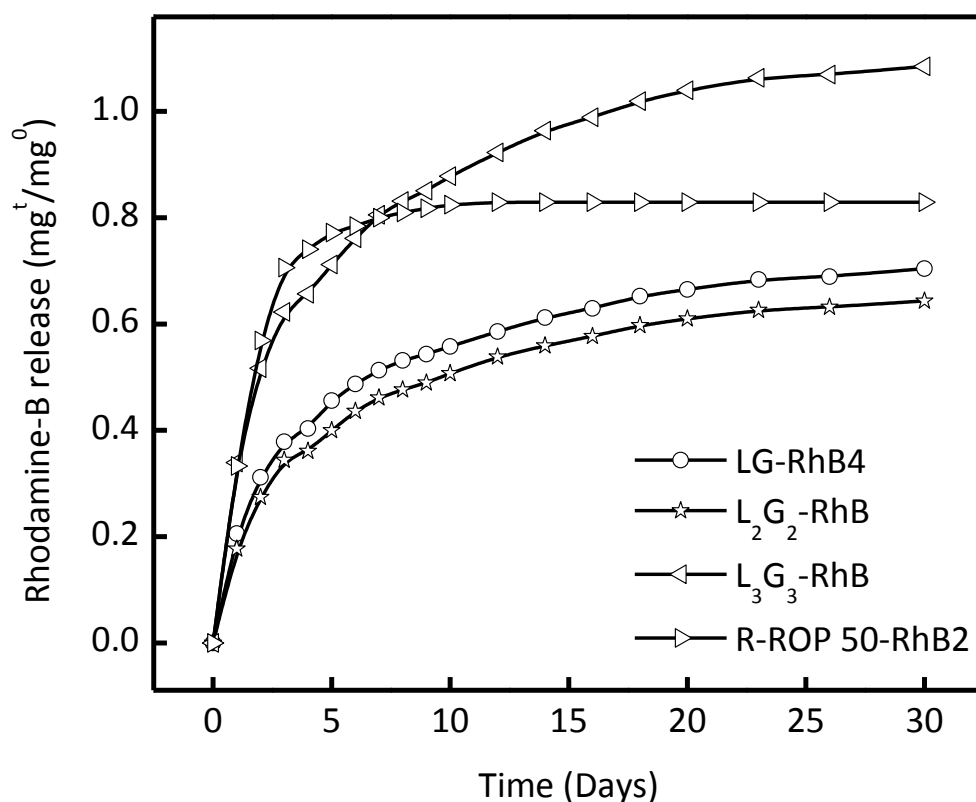
<sup>a</sup>Molecular weights and polydispersity indices determined by SEC in THF vs. polystyrene standards. <sup>b</sup>Based on 200 mg polymer sample size, calculated according to Equation 2.

From the loading capacity and loading rates of RhB in these PLGAs it appears as if **LG-RhB4** and **L<sub>2</sub>G<sub>2</sub>-RhB** have similar affinity for RhB (ca. 5%). **L<sub>3</sub>G<sub>3</sub>-RhB**, however, exhibits a loading rate of only 3.2%. As the **L<sub>3</sub>G<sub>3</sub>-RhB** copolymer is arguable closer in structure to the random copolymer, this behavior is puzzling. The only prominent difference between these samples is the higher molecular weight of the **L<sub>3</sub>G<sub>3</sub>-RhB** relative to the others, but as it is an increase of only about 25% it is difficult to assign this difference as the primary factor.

The release profiles of RhB are plotted in Figure 24. **L<sub>2</sub>G<sub>2</sub>-RhB** and **LG-RhB4** have very similar release profiles. During the first day of release, ~ 20% of RhB released from these PLGA carriers. The half-released time of **LG-RhB4** is 6.5 days and **L<sub>2</sub>G<sub>2</sub>-RhB** is 9.5 days. Neither of the profiles shows a plateau during release. Both of them exhibit steady release rates from the 3<sup>rd</sup> day to the end. And at the end of the release experiment, **LG-RhB4** shows a 70% of RhB released and **L<sub>2</sub>G<sub>2</sub>-RhB** shows a 64% of RhB released in total.

The release profile of **L<sub>3</sub>G<sub>3</sub>-RhB** is distinctly different from both the other sequenced copolymers and the random control. Release of RhB is very fast in the first day from **L<sub>3</sub>G<sub>3</sub>-RhB**

that more than 30% is released. Then a rapid but steady release profile can be observed. The half-released time of **L<sub>3</sub>G<sub>3</sub>-RhB** is the same as that of **R-ROP 50-RhB3**, but no release plateau is observed. Also, the total release is higher than that observed for the random copolymers, which does not seem reasonable. We think that it is likely that the **L<sub>2</sub>G<sub>2</sub>-RhB** data are accurate and that there is a significant problem, likely in the determination of the initial loading, for the **L<sub>3</sub>G<sub>3</sub>-RhB** sample. Without repeating the LG, L<sub>2</sub>G<sub>2</sub>, L<sub>3</sub>G<sub>3</sub> series, however, we cannot be confident in our analysis.



**Figure 24.** Release of Rhodamine-B from PLGA microparticles immersed in a pH 7.4 buffer at 37 °C: 30 day study focusing on the effect of sequence.

### 3.5 CONCLUSIONS

Hydrolytic degradation rates for repeating sequence PLGAs are found to exhibit dramatically different hydrolysis behaviors compared with random analogues. Lactic acid release rates were also found to strongly depend on the monomer sequence. Thermal property changes during hydrolysis shows uniform degradation of polymer chains in sequence controlled PLGAs. Preliminary *in vitro* RhB release profiles shows that using sequenced PLGAs as carriers could effectively reduce the burst release and extend the release time of low molecular weight drug model compare to using random PLGAs. Repeating sequenced PLGAs are therefore good candidates for applications that depend on a steady and controllable degradation rate.



## 4.0 PROSPECTUS

This study shows that hydrolytic degradation behaviors and rhodamine-B release profiles are related to monomer sequences of PLGA. PLGA with sequence control will be employed in biomedical applications to 1) achieve a controlled release of drug by a gradual degradation of delivery system, 2) maintain mechanical properties by slower loss of molecular weight and 3) avoid slow-degradation residue because of homogenous degradation. Therefore, based on the preliminary results we have in this dissertation, better understanding of sequence-property relationship is needed.

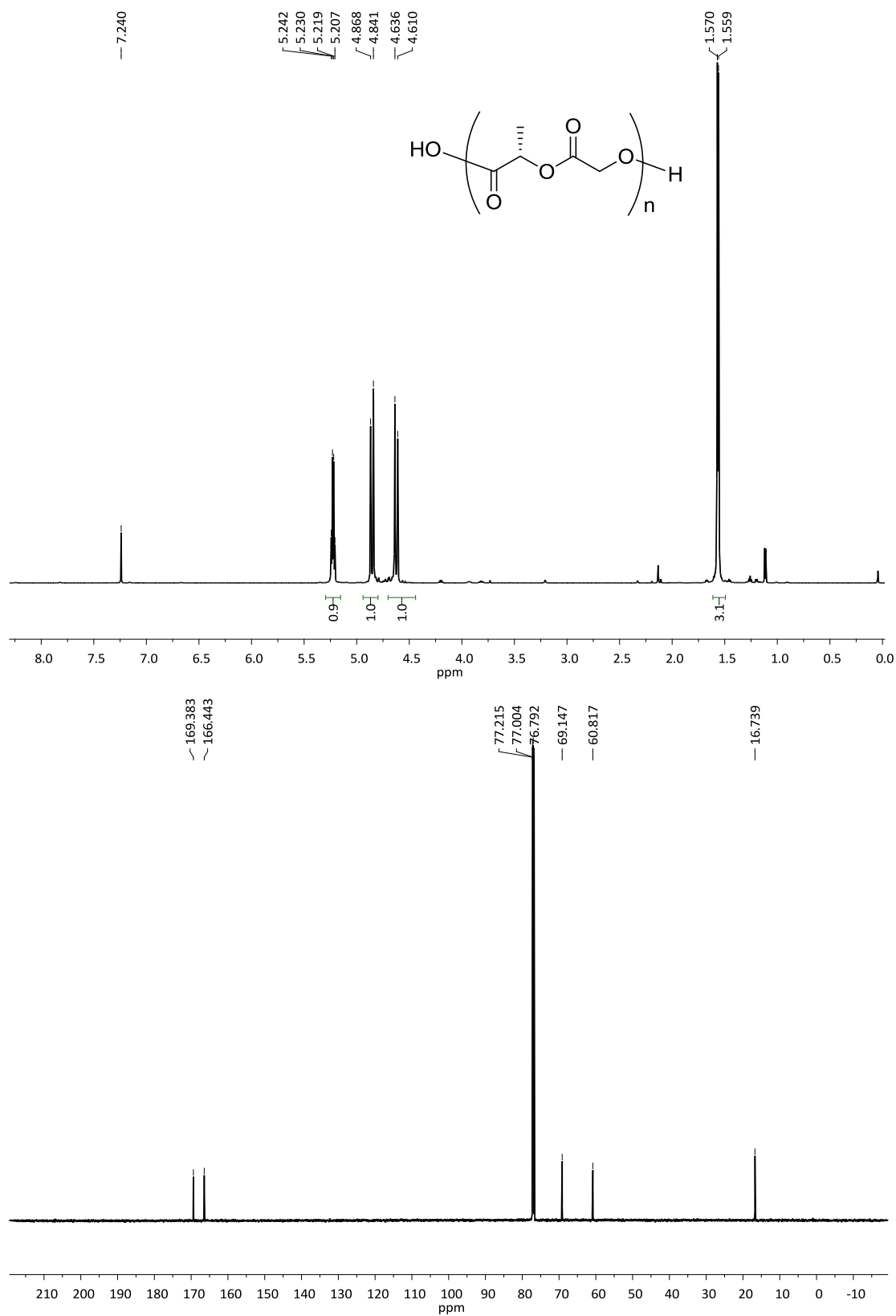
First, release profiles of different types of drugs, such as lipophilic drugs, protein and DNA, in sequenced PLGA microparticles should be studied both *in vitro* and *in vivo*. Sequenced PLGA microparticles used in drug delivery system as carriers should be evaluated.

Second, a comprehensive study of hydrolytic degradation behavior of sequenced PLGA in bulk morphology should be studied. The slow loss of molecular weight shows sequenced PLGA has potential to slow the uptake of water and bear a slower mass loss in bulk. This will extend its application in tissue engineering area.

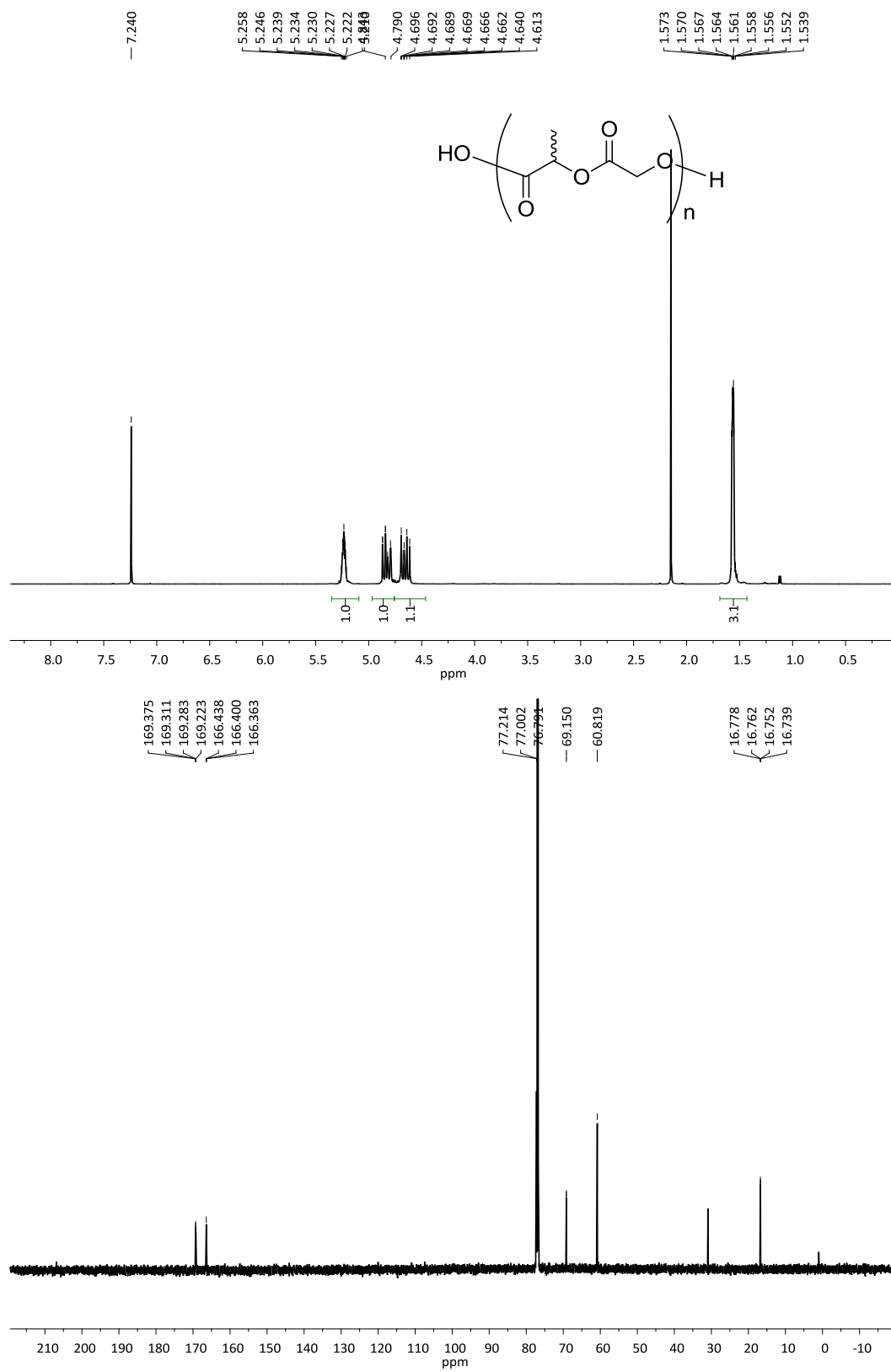
A mathematic model then should be build up to construct relations between degradation behavior and sequence of PLGA. The model will offer a guidance to utilize different sequenced PLGA in multiple applications, such as custom designed drug release profiles and degradable scaffolds in cell therapies.<sup>139</sup>

## **APPENDIX A**

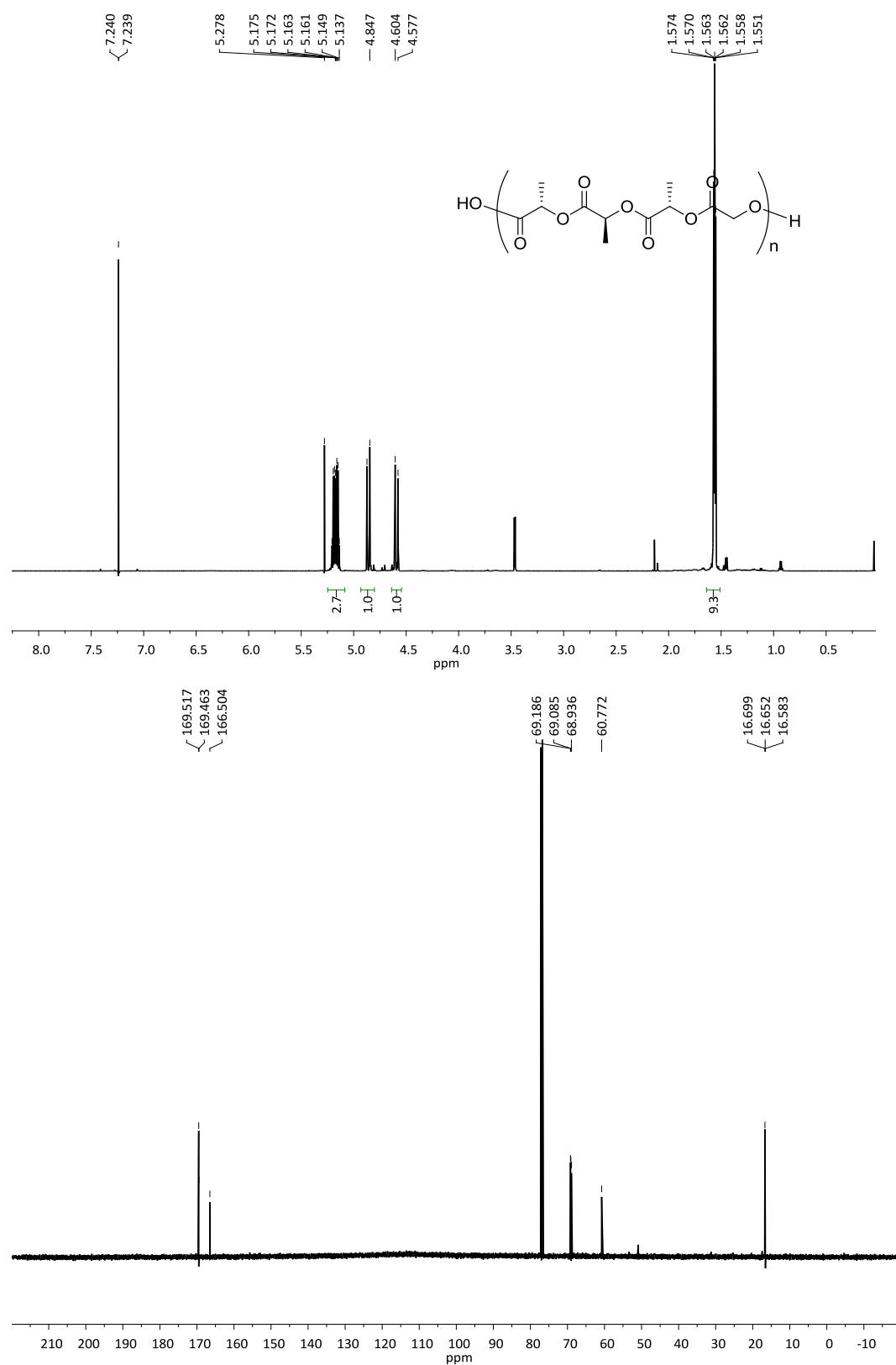
### **NMR SPECTRA OF NEW COMPOUNDS AND POLYMERS**



**Figure 25.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of Poly LG

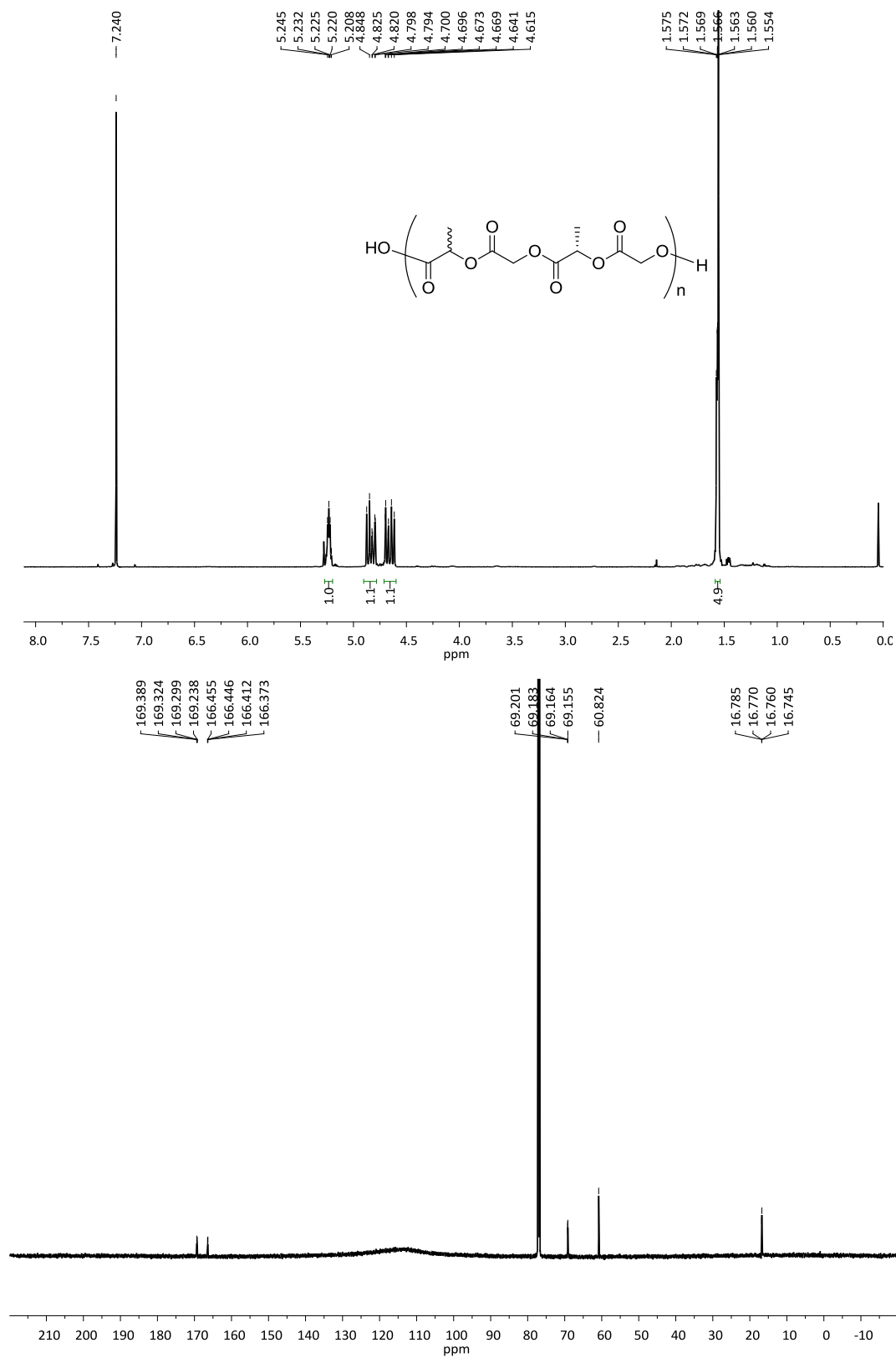


**Figure 26.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Poly L<sub>rac</sub>G

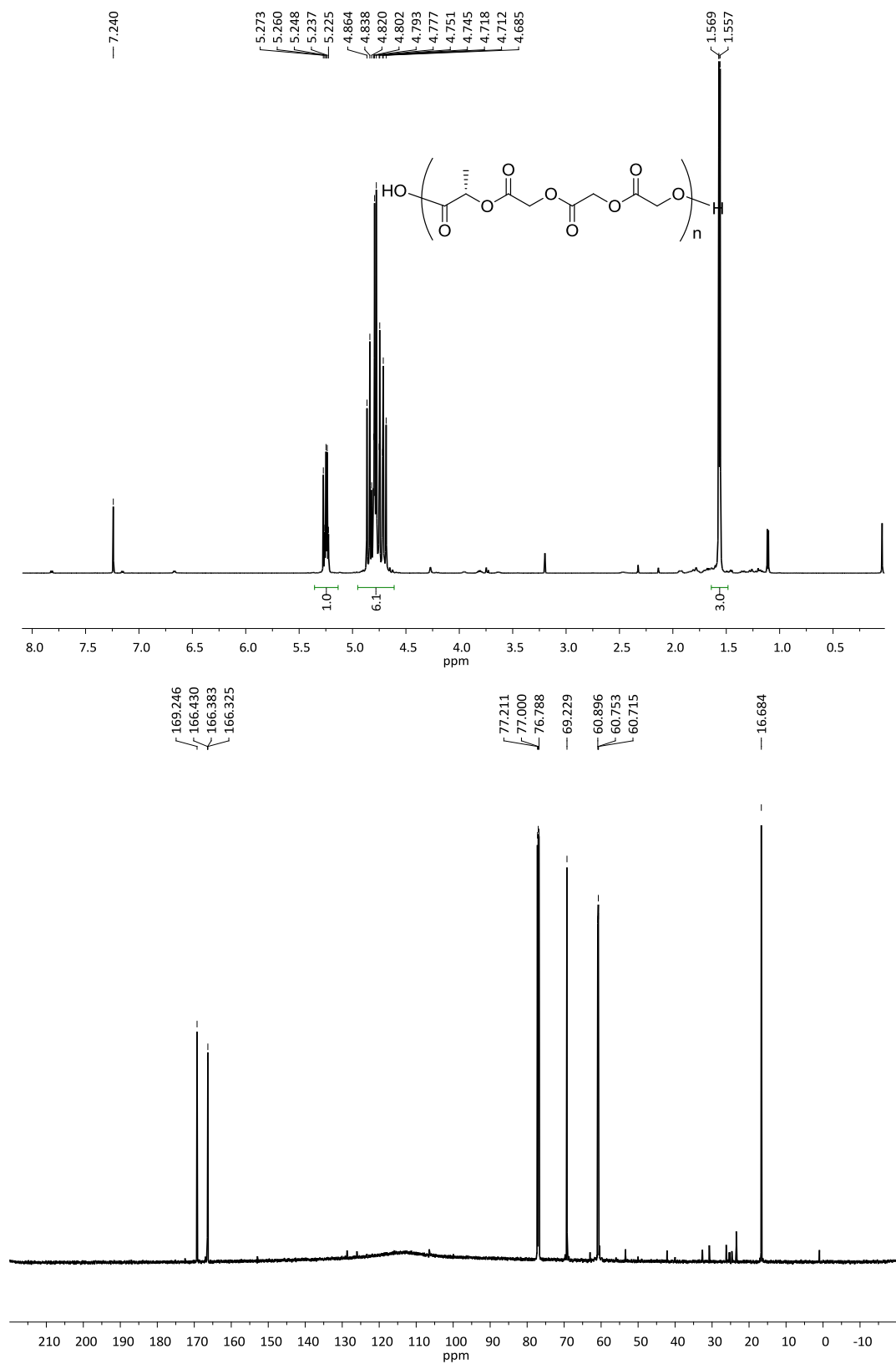


**Figure 27.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Poly LLLG





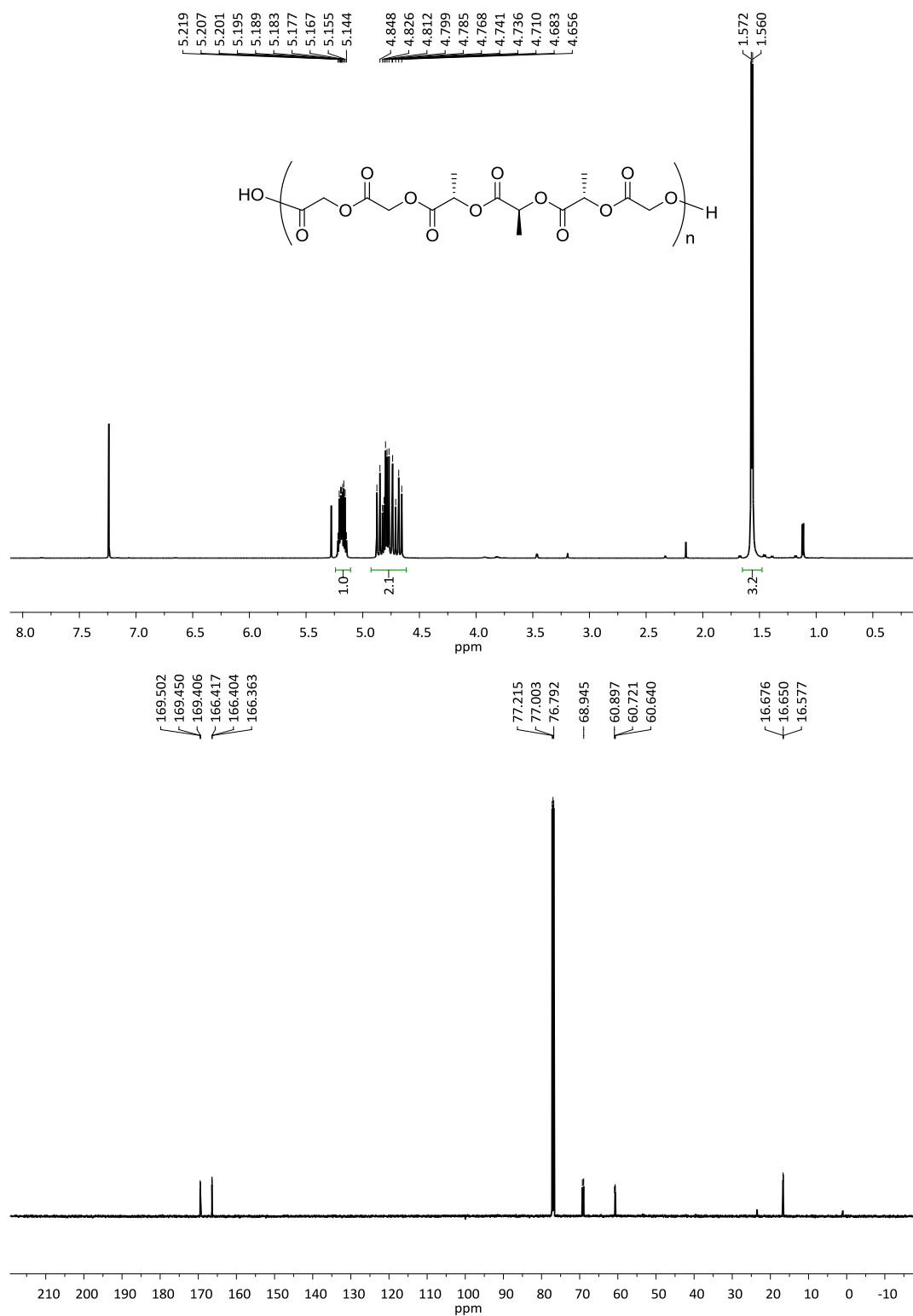
**Figure 29.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Poly L<sub>rac</sub>GLG



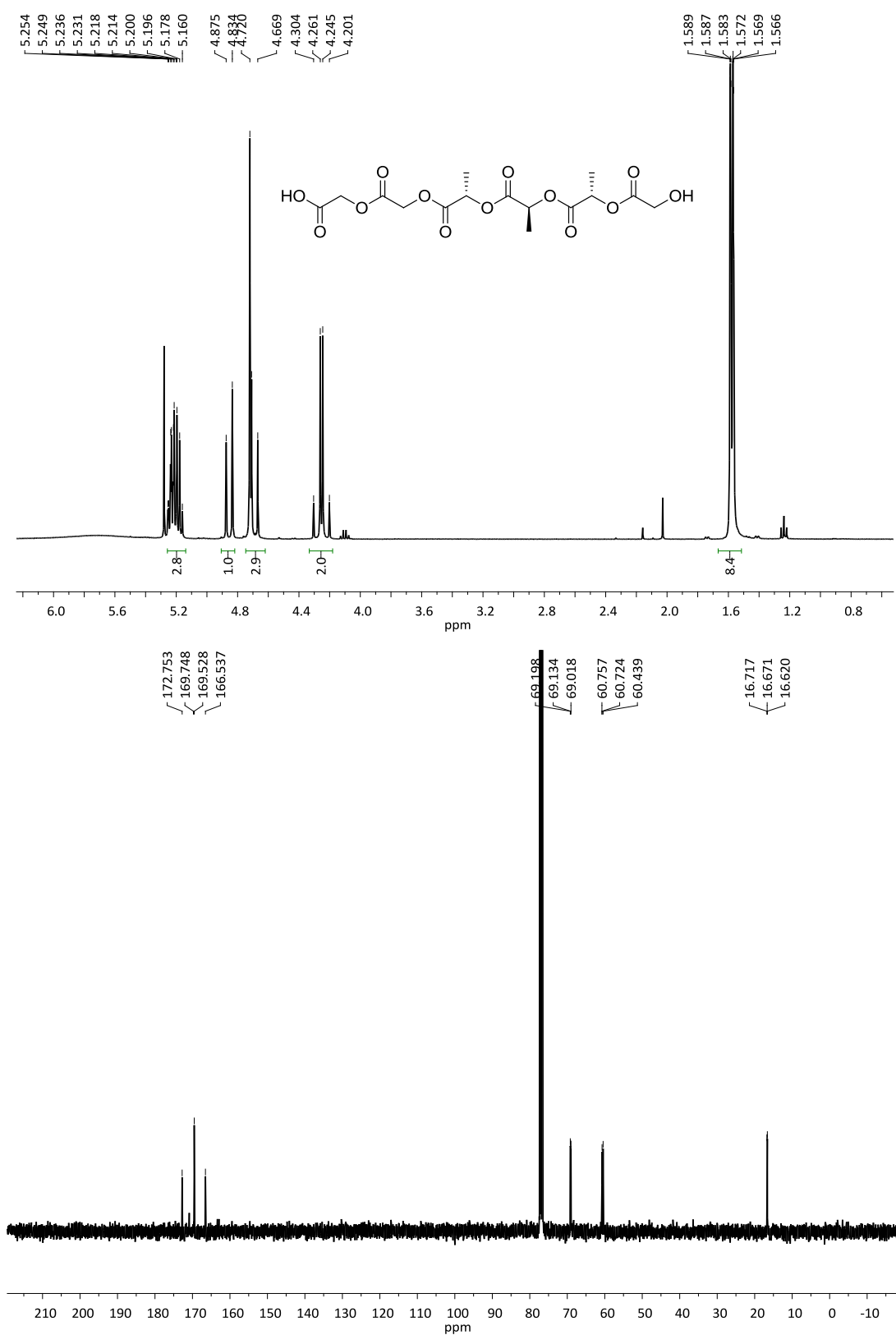
**Figure 30.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Poly LGGG





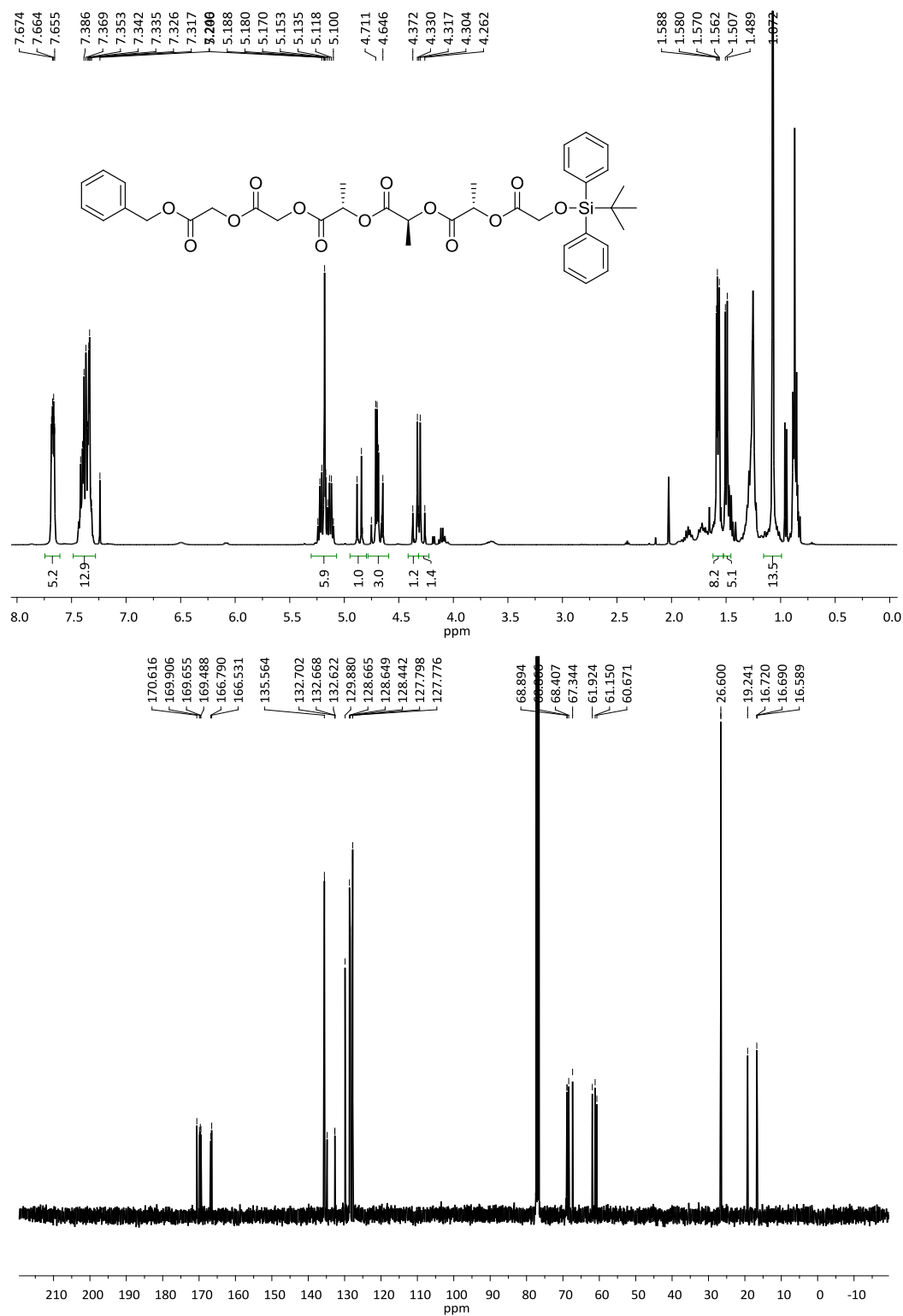


**Figure 32.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Poly GLLLG

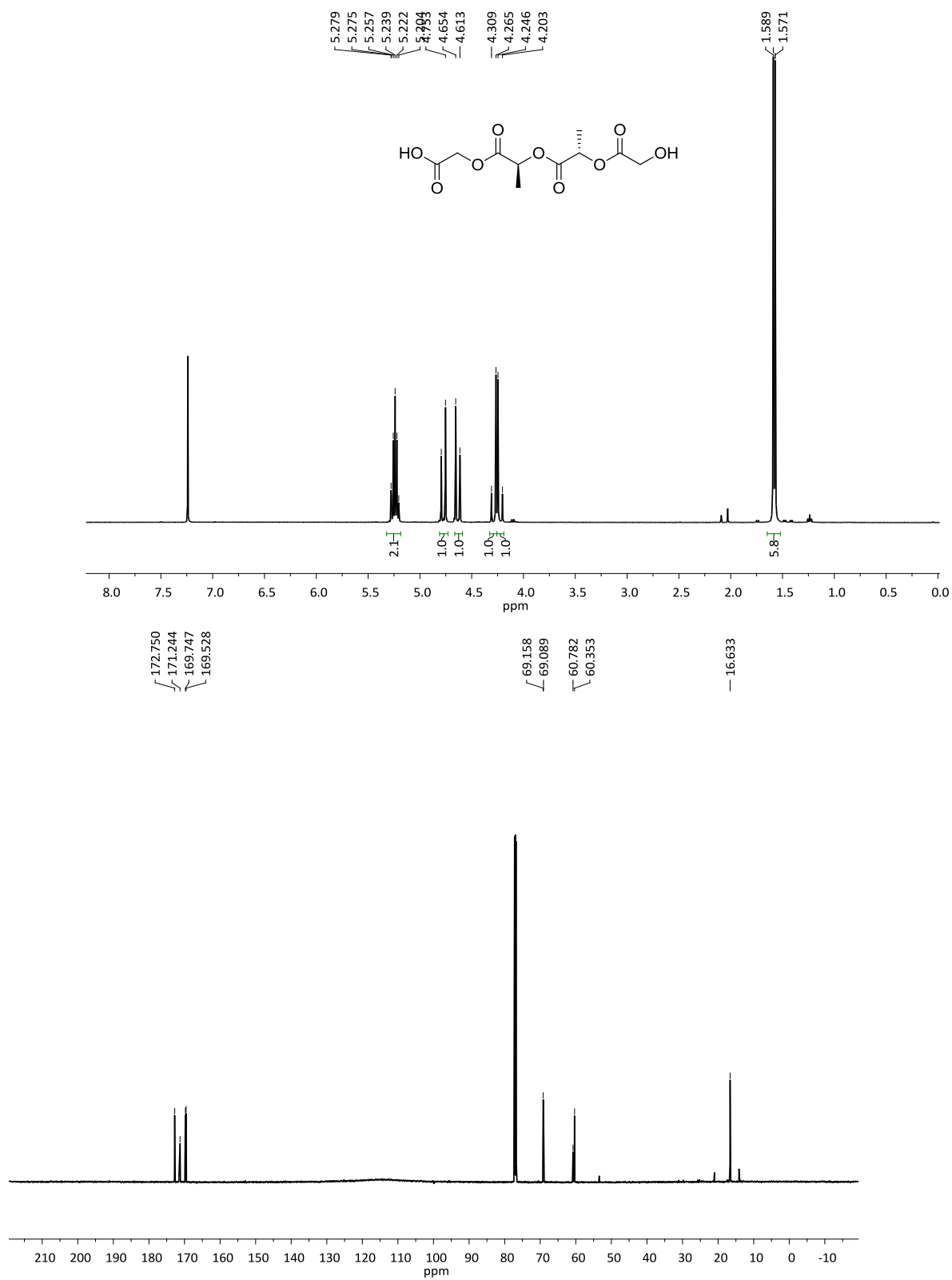


**Figure 33.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of GLLLLG

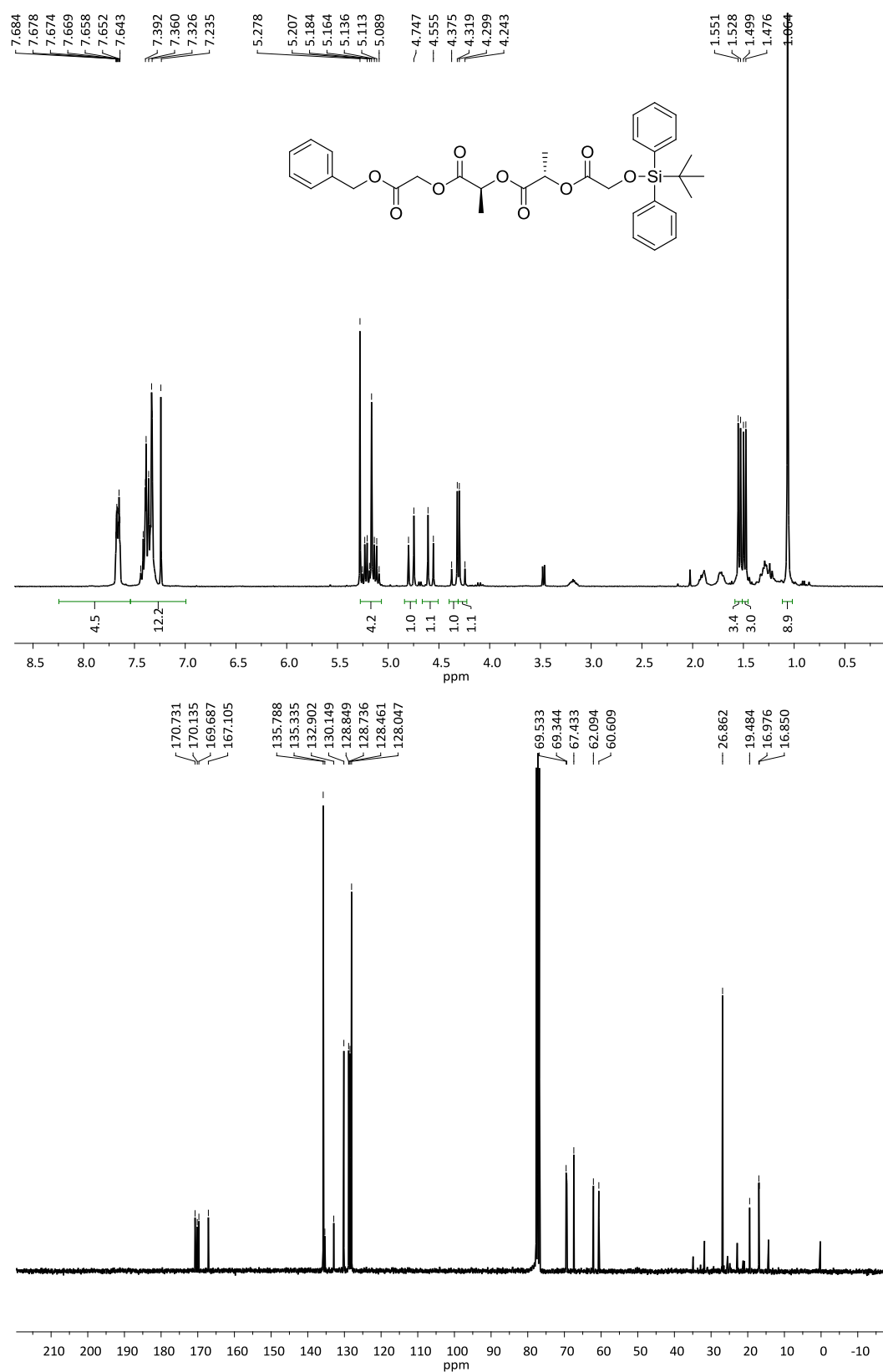




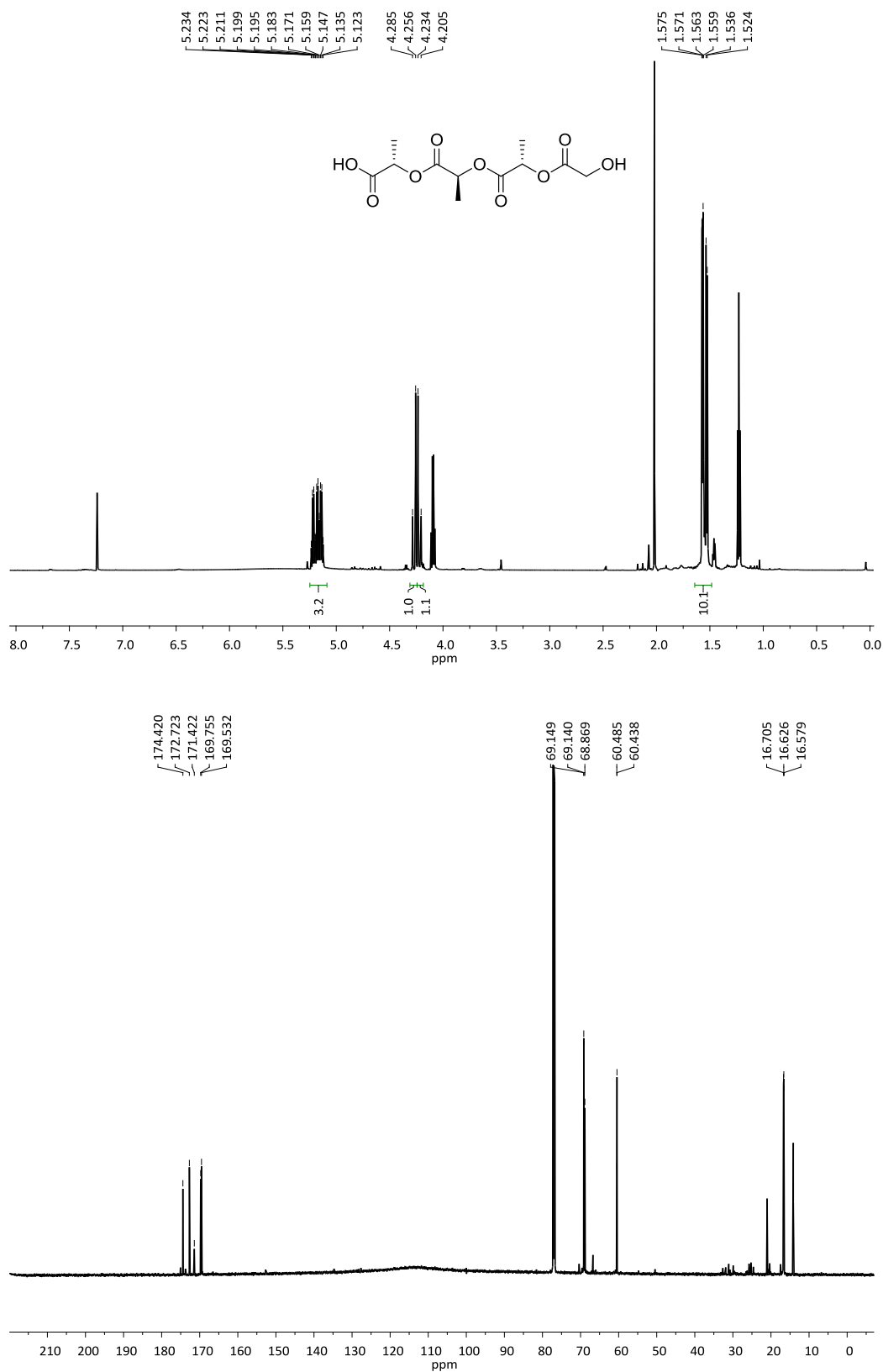
**Figure 35.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-GLLLLG-Si



**Figure 36.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of GLLG



**Figure 37.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-GLLG-Si



**Figure 38.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of LLLG



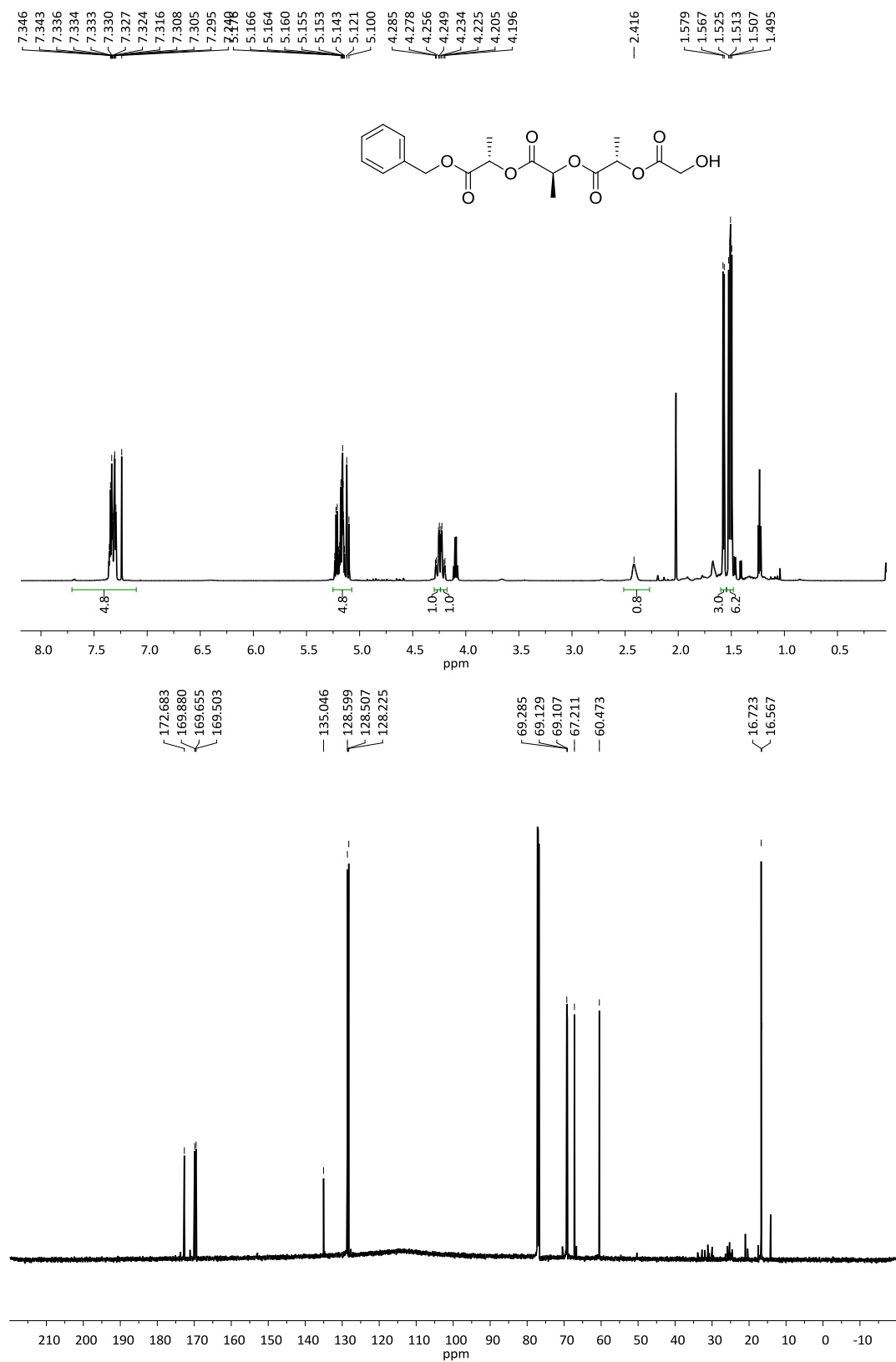
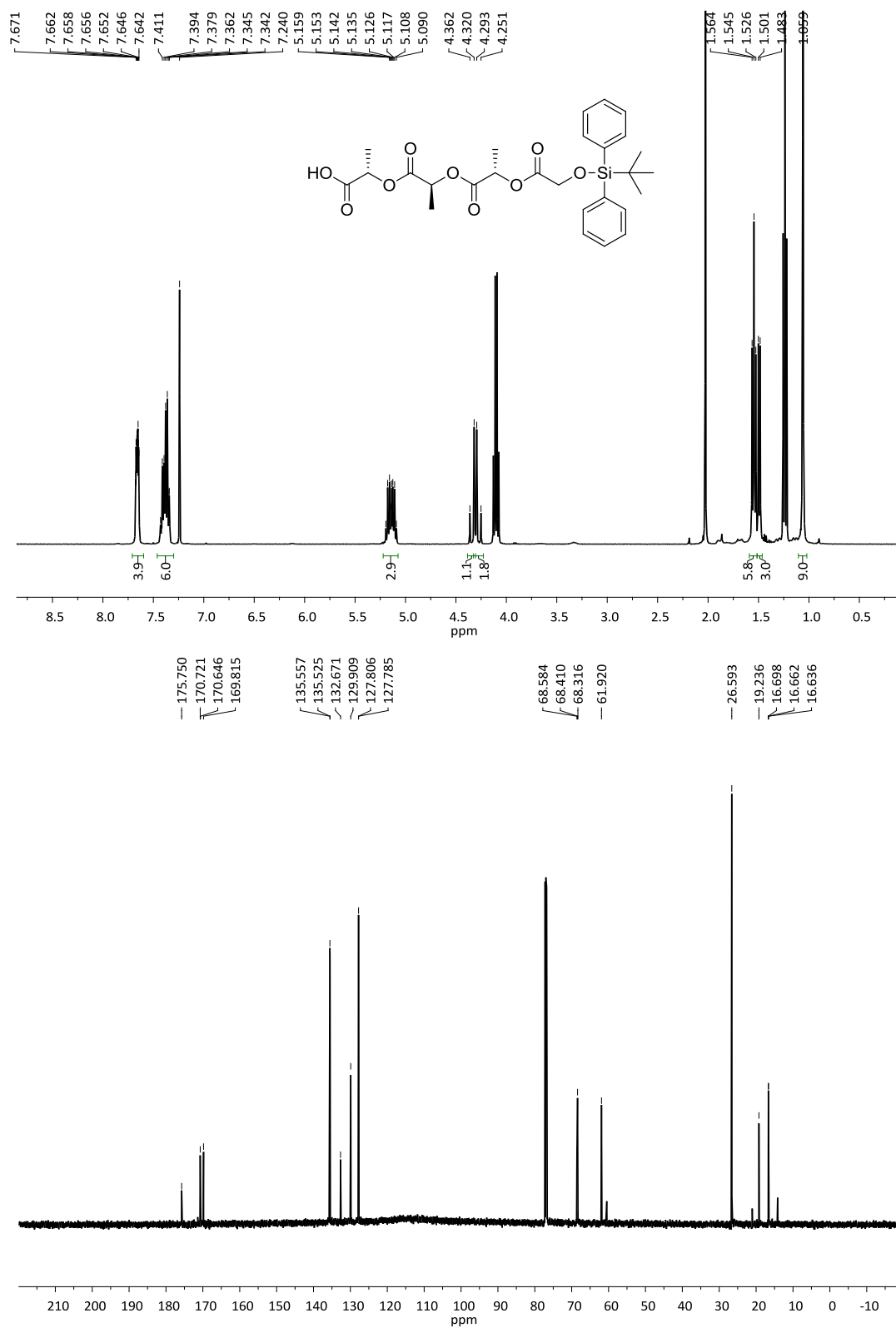
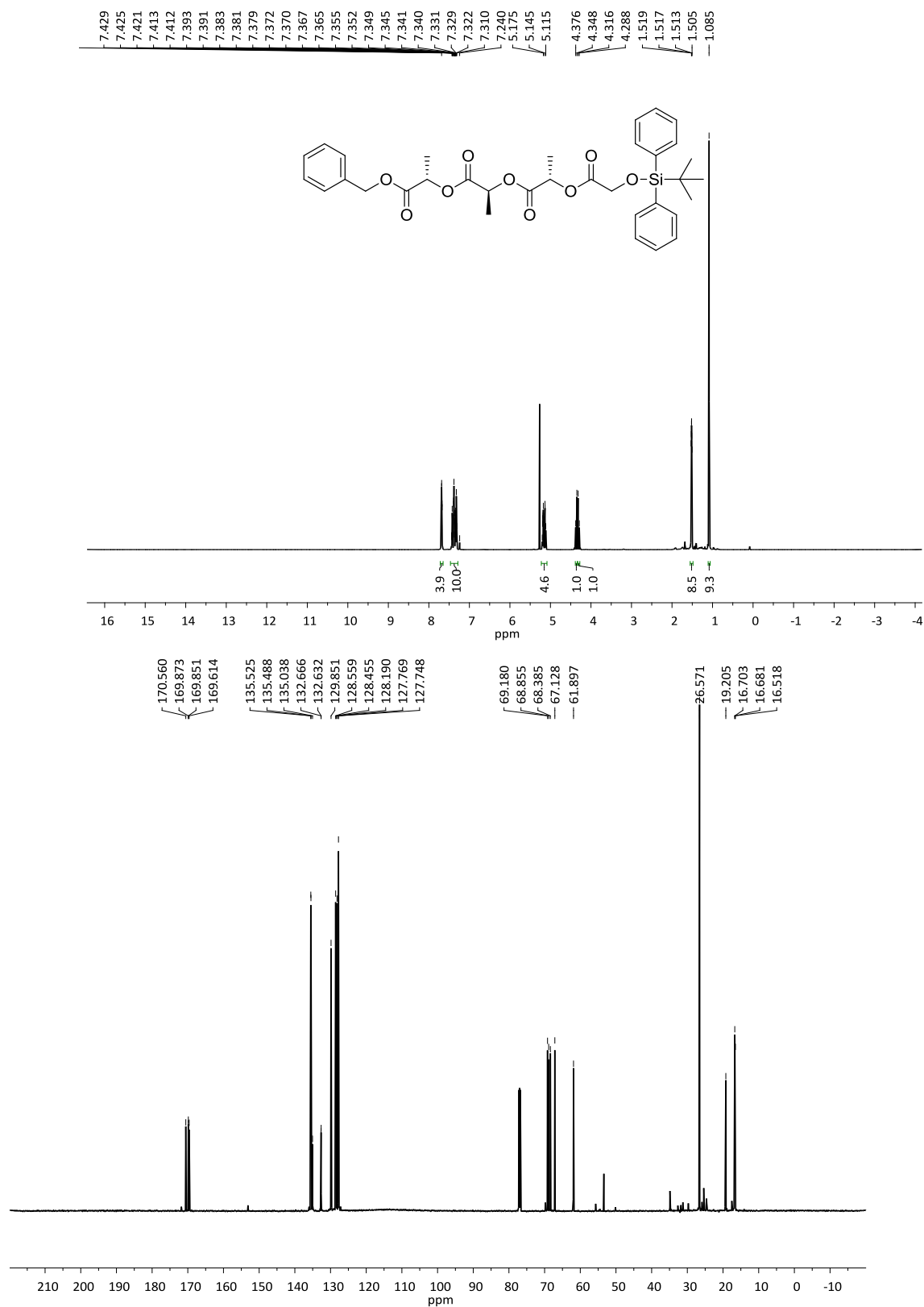


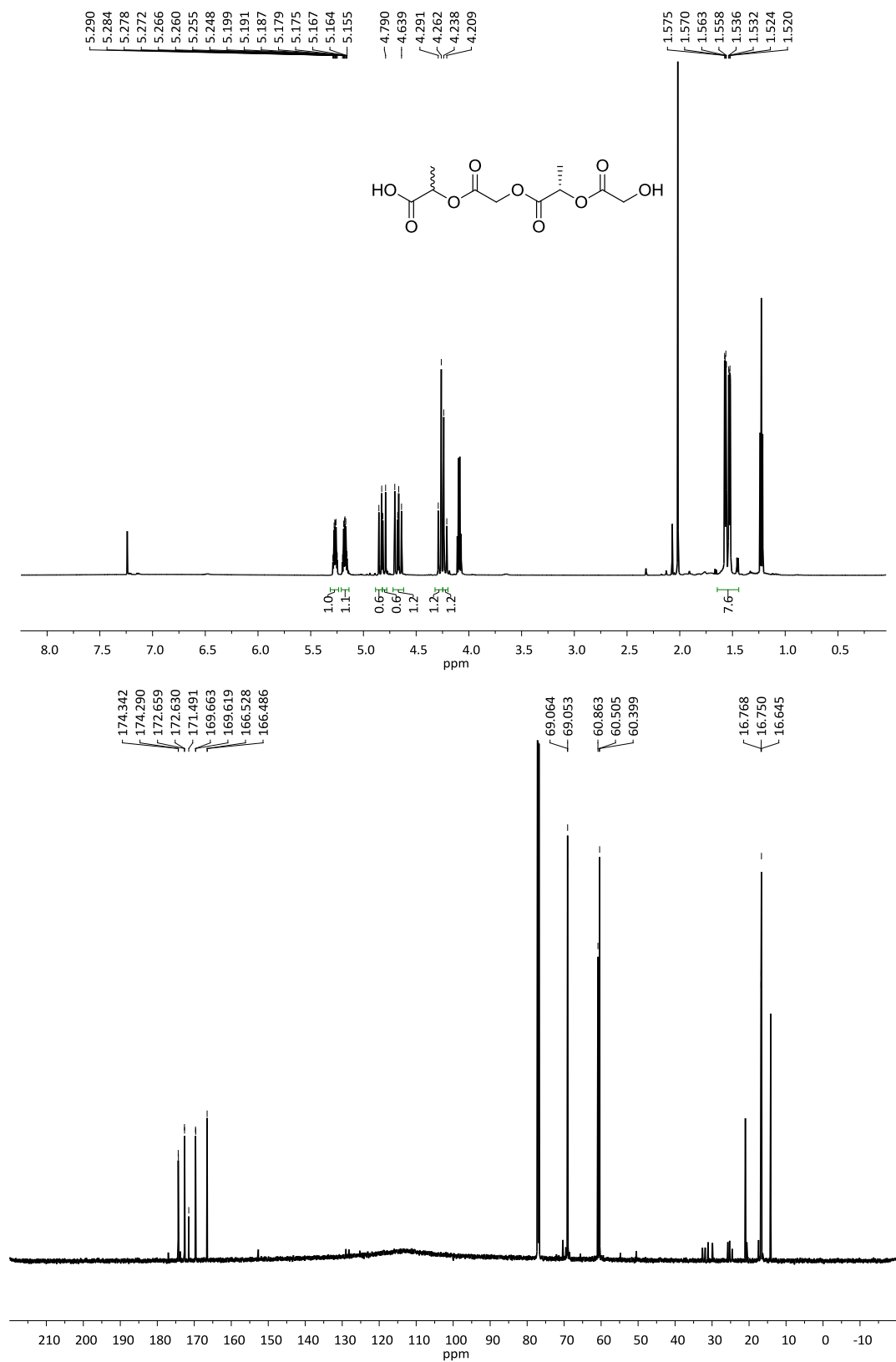
Figure 39. <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-LLLG



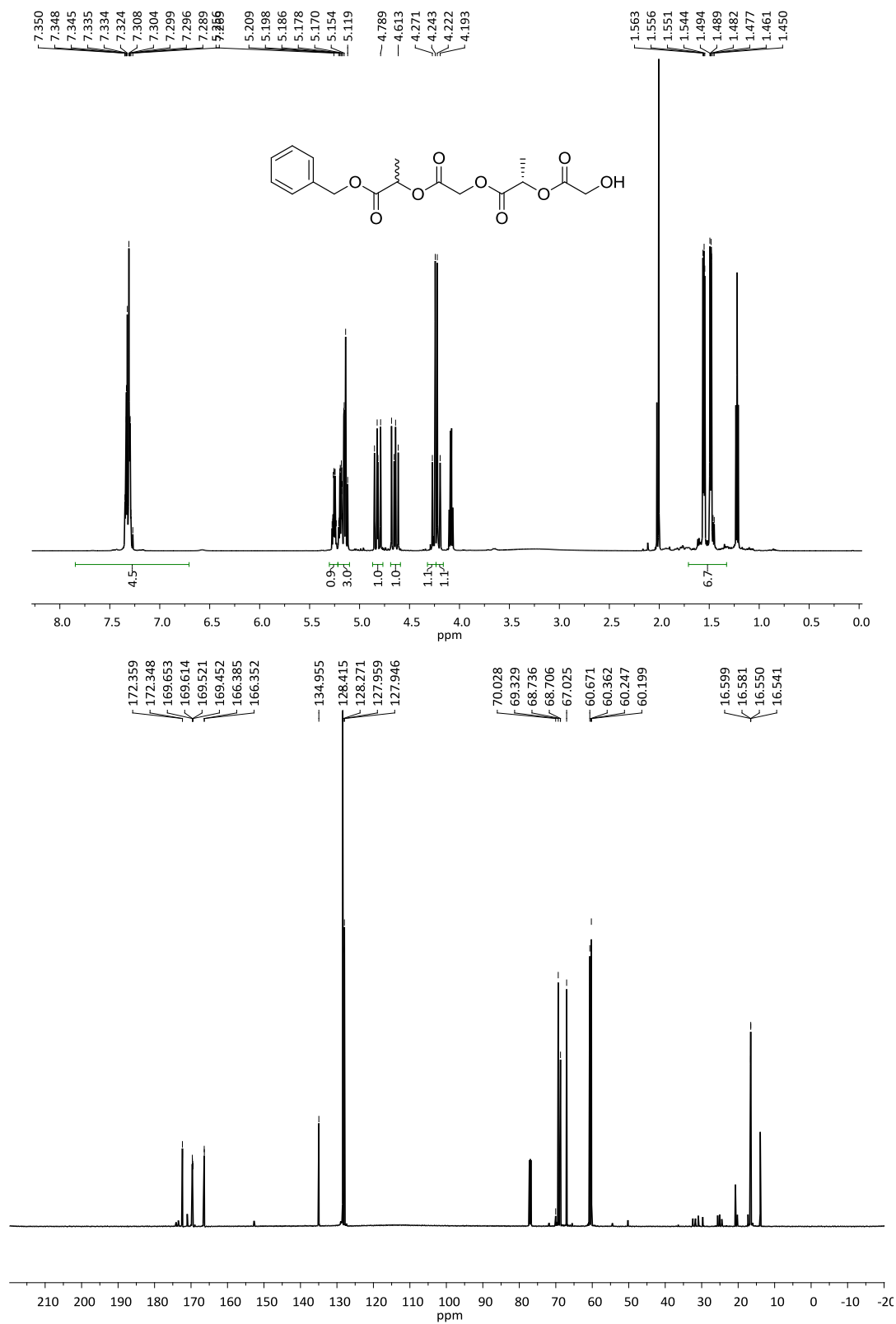
**Figure 40.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of LLLG-Si



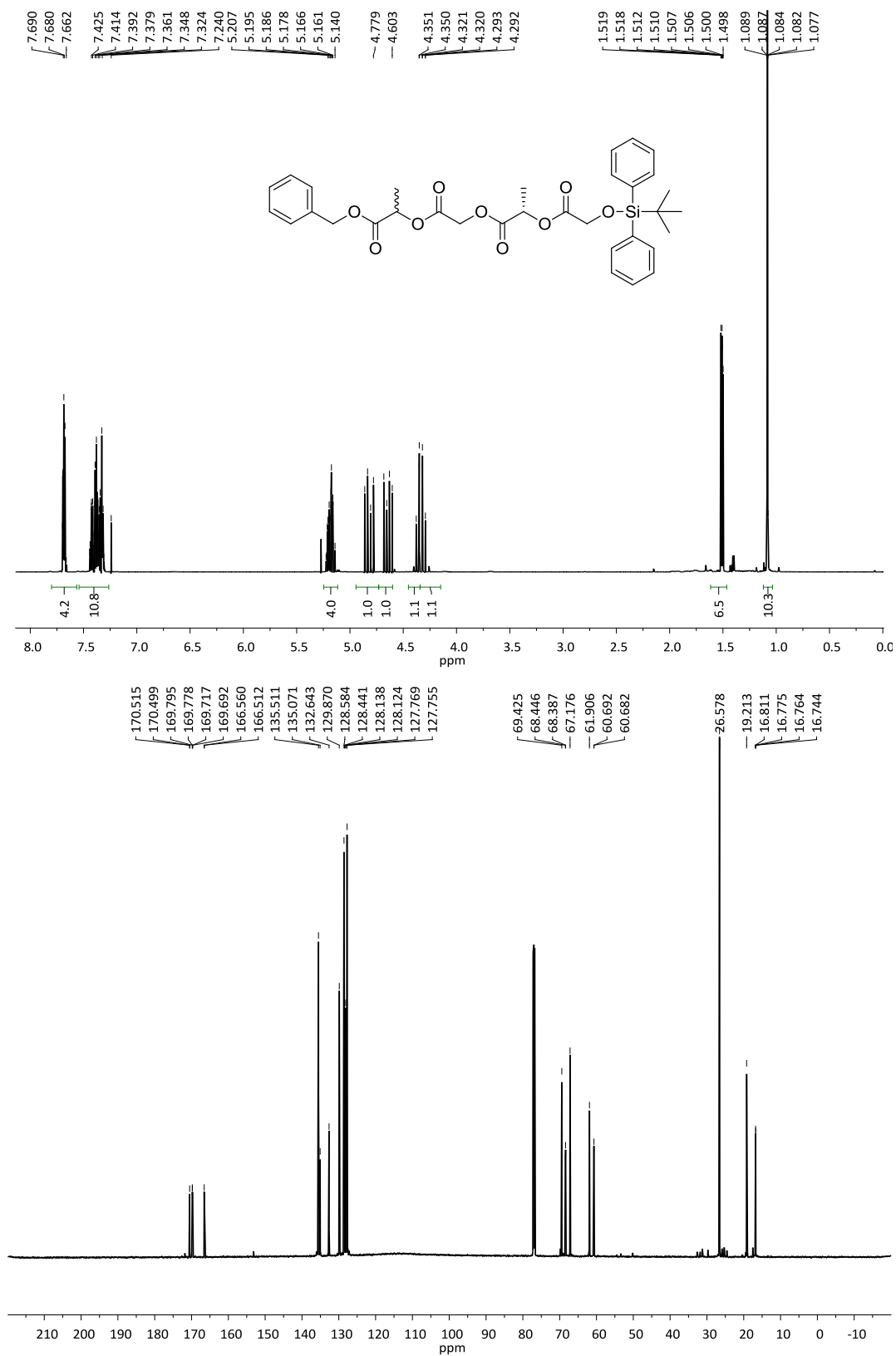
**Figure 41.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-LLLG-Si



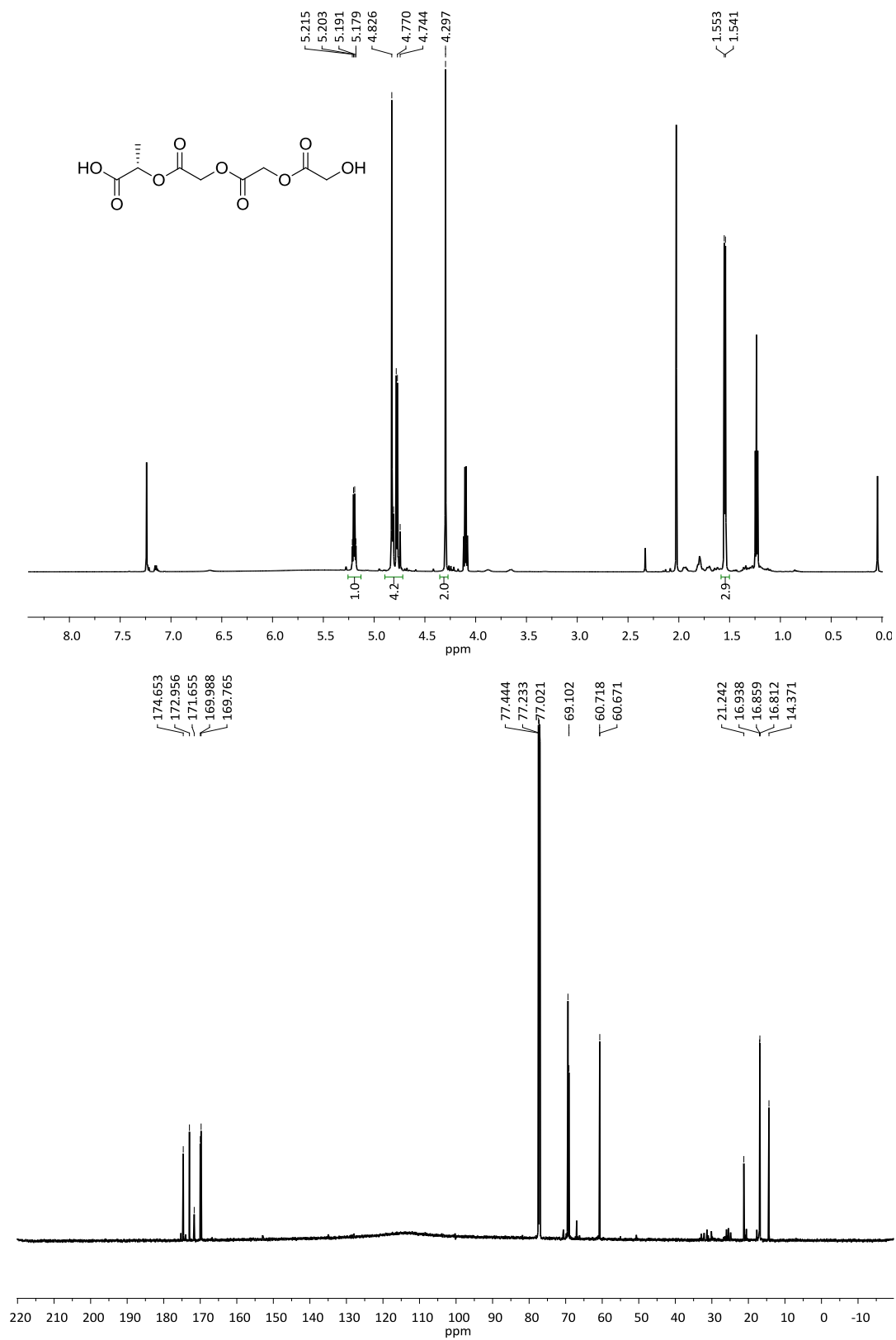
**Figure 42.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of  $L_{rac}\text{GLG}$



**Figure 43.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-L<sub>rac</sub> GLG



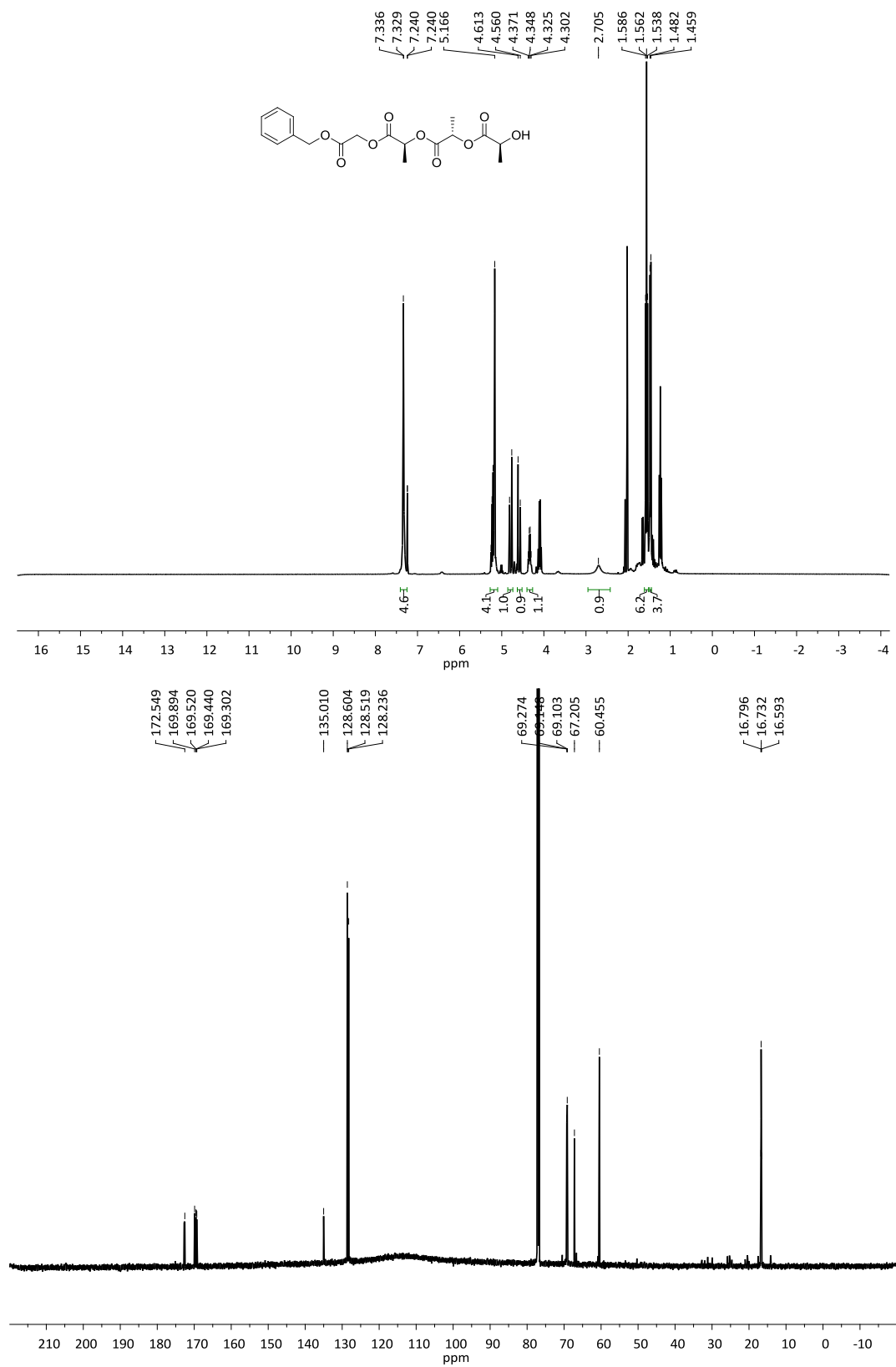
**Figure 44.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-L<sub>rac</sub>GLG-Si



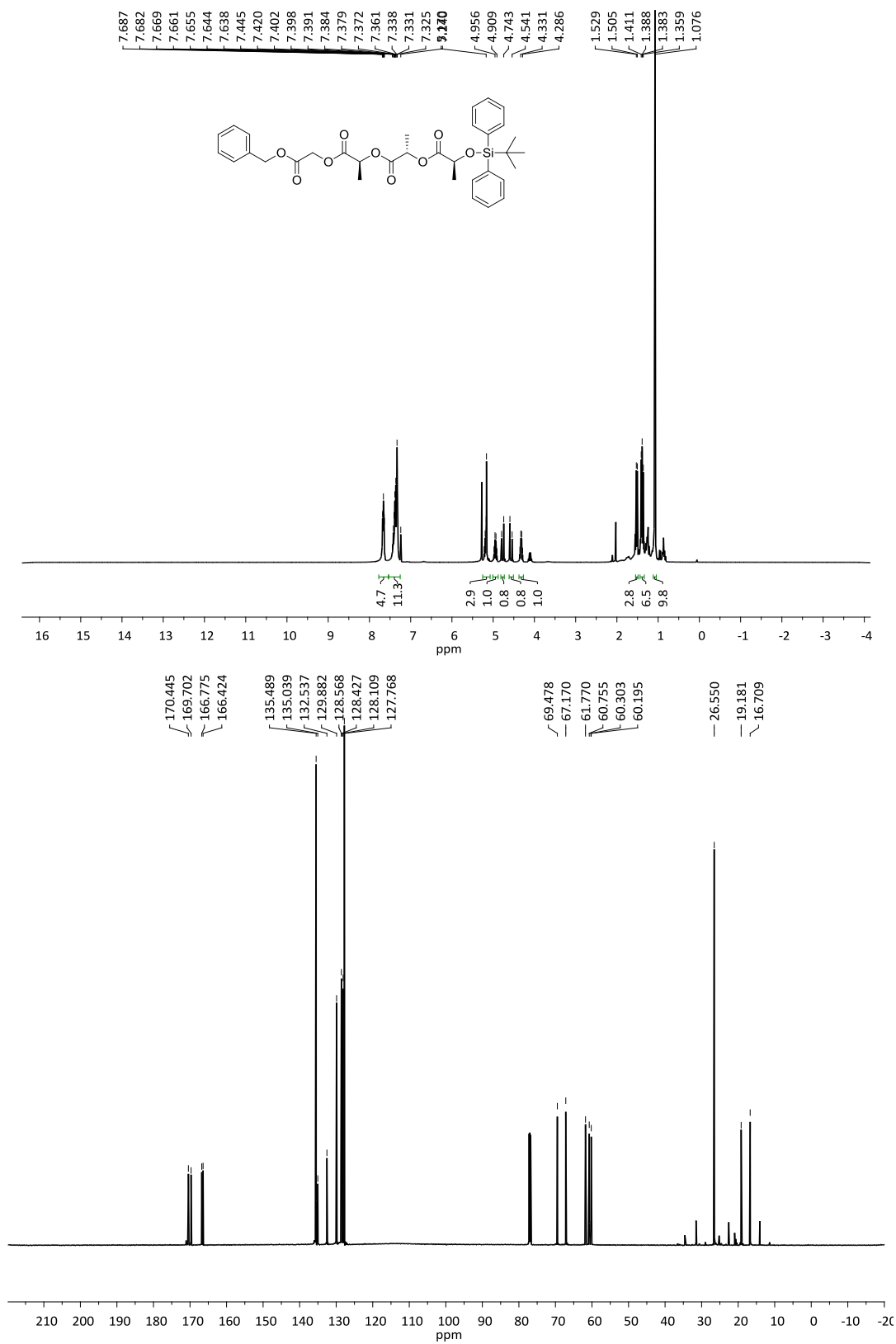
**Figure 45.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of LGGG







**Figure 47.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-GLLL



**Figure 48.** <sup>1</sup>H and <sup>13</sup>C NMR spectra of Bn-GLLL-Si

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